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# Microbial communities in a former pilot-scale uranium mine in Eastern Finland – Association with radium immobilization

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## HIGHLIGHTS

- Bacterial, fungal and archaeal communities characterized in a former uranium mine.
- Several microbial groups found, well adapted to the elevated radiation in the area.
- Especially bacterial and archaeal communities affected by <sup>226</sup>Ra.
- pH and sulfate concentrations appeared to control the solubility of radium.
- *Pseudomonas* strains were shown to take up Ra and to leach Ra from soil.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The bacterial, fungal and archaeal communities were characterized in 17 top soil organic and mineral layer samples and in top sediment samples of the Paukkajänvaara area, a former pilot-scale uranium mine, located in Eno, Eastern Finland, using amplicon sequencing and qPCR. Soil and sediment samples were in addition analyzed for radium (<sup>226</sup>Ra), sulfate (SO<sub>4</sub><sup>2−</sup>), nitrate (NO<sub>3</sub><sup>−</sup>) and phosphate (PO<sub>4</sub><sup>3−</sup>) concentrations. New bacterial strains, representing *Pseudomonas* spp., were isolated from the mine and reference area and used in laboratory experiments on uptake and leaching of radium (Ra). The effect of these strains on the sulfate leaching from the soil samples was also tested in vitro. Between  $6 \times 10^6$  and  $5 \times 10^8$  copies g<sup>−1</sup> DW (dry weight) of bacterial 16S rRNA genes,  $5 \times 10^5$ – $1 \times 10^8$  copies g<sup>−1</sup> DW archaeal 16S rRNA genes and  $1 \times 10^5$ – $1 \times 10^8$  copies g<sup>−1</sup> DW fungal 5.8S rRNA genes were detected in the samples. A total of 814, 54 and 167 bacterial, archaeal and fungal genera, respectively, were identified. *Proteobacteria*, *Euryarchaeota* and *Mortierella* were the dominant bacterial, archaeal and fungal phyla, respectively. All tested *Pseudomonas* spp. strains isolates from Paukkajänvaara removed Ra from the solution, but the amount of removed Ra depended on incubation conditions (temperature, time and nutrient broth). The highest removal of Ra (5320 L/kg DW) was observed by the *Pseudomonas* sp. strain T5-6-I at 37 °C. All *Pseudomonas* spp. strains decreased the release of Ra from soil with an average of 23% while simultaneously increasing the concentration of SO<sub>4</sub><sup>2−</sup> in the solution by 11%. As *Pseudomonas* spp. were frequent in both the sequence

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data and the cultures, these bacteria may play an important role in the immobilization of Ra in the Paukkajanvaara mine area.

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## 1. Introduction

Microorganisms have essential roles in soil biogeochemical cycles and microbiota readily respond to the changes occurring in the soil conditions due to toxic compound, including radionuclides, pollution (Marin et al., 2005; Tejada et al., 2008; Marcin et al., 2013; Li et al., 2013). On the abandoned mine sites, the mobilization of radionuclides from mill tailings and other mine by-products may be amplified because of acidic runoffs (Acid Mine Drainage, AMD) (Robb, 1994; Mason, 2002), typical for sulfide ores in the presence of water and chemolithotrophic microorganisms. Subsequently, radionuclides, including radium (Ra), may be leached from minerals over time and be released into the environment, contaminating local water and soil resources (Bhattacharyya, 1998; Darko et al., 2009). In addition to initial radionuclide concentrations and chemical forms of radionuclides in the ore, mining processes, such as the selection of the extraction procedure and enrichment methods, may also contribute to the increased migration of radionuclides into the environment (Paschoa, 1998). Typically, at the end of the mining activities, only the waste rock and mill tailing storage areas and quarries will remain in the area. Despite the after-care technologies used in the mining industry, increased radionuclide concentrations are found in many former mining areas, like in Paukkajanvaara (Finland), former pilot scale uranium (U) mine area sediments (Tuovinen et al., 2016).

The level of radiological exposure of humans and other biota to radionuclides from former mine areas depends on the environmental dispersion of naturally occurring radionuclides, including Ra, as well as their transfer pathways. These transfer pathways include the release of radionuclides from waste rock and tailings to soil, sediments and water reservoirs as well as uptake by plants and animals from soil (Carvalho et al., 2014). In addition, radionuclides may migrate with the groundwater flow (Saunders et al., 2016).

Many of these transfer pathways include microbiological steps and mediators, which are however still largely unknown. Therefore, the characterization of microorganisms in such radionuclide-polluted environments is important and improves our understanding of the impacts of radionuclides on microbial ecology and evolution and vice versa. This also helps in the understanding of the mechanisms of microbial radionuclide tolerance (Méndez-García et al., 2015; Chen et al., 2016; Huang et al., 2016). Previous microbial population analyses have e.g. shown modifications of bacterial communities in response to changes in pH (Chen et al., 2016; Kuang et al., 2016), which can also be expected for changing radionuclide and metal concentrations.

In recent years, microbial communities have been examined using culture-independent molecular techniques e.g. from mine tailings, radioactive oil waste and natural uranium-rich soils (e.g. Huang et al., 2016; Xie et al., 2009; Galitskaya et al., 2015; Mondani et al., 2011). However, the majority of these studies have concentrated on bacterial communities and very few of the previous studies have used amplicon sequencing and qPCR approaches to examine in addition to bacteria, also archaeal and fungal communities associated with radionuclides in mine areas. Comprehensive information on soil bacterial, archaeal and fungal community composition and behavior is however essential for our understanding of the links between biotic and abiotic transfer processes of radionuclides in the environment (Prenafeta Boldú et al., 2014). Furthermore, to our knowledge, none have characterized the uptake of Ra by bacteria isolated from a uranium mine area, and in addition combined it to direct amplicon sequencing data of the microbial communities from the

same area. In fact, previous studies on Ra uptake and biosorption are particularly scarce and Ra uptake/biosorption has been previously reported only in few bacteria, including *Citrobacter freundii*, *Corynebacterium* sp. strain T-A, *Chromobacterium* sp. strain A-E and *Chryseobacterium* sp. strain CH-G (Satvatmanesh et al., 2003) as well as *Pseudomonas aeruginosa* (Strandberg et al., 1981), and *P. fluorescens* and *Streptomyces niveus* (Tsezos and Keller, 1983). The abandoned mine areas afford us a unique opportunity to examine microbial populations subjected to long-term radiation exposure and possibly enables us to identify microorganisms well adapted to the presence of radionuclides. To address this, in this study the bacterial, archaeal and fungal communities of soil and sediment samples collected from the Paukkajanvaara former pilot-scale uranium mine area were analyzed using an amplicon sequencing approach combined with isolated bacterial strains and batch uptake and leaching experiments of Ra using these bacteria and mine area samples.

## 2. Materials and methods

### 2.1. Paukkajanvaara site

Paukkajanvaara former pilot scale U mine site is located in the municipality of Eno in Northern-Karelia, Eastern Finland, approximately 50 km from the city of Joensuu. Eastern Finland belongs to the southern boreal forest zone. The climate in the area is sub-arctic continental, which is characterized by warm summers and cold winters. The average temperature of the warmest and coldest months (July and February, respectively) is 21 °C and −13 °C, respectively ([www.uef.fi](http://www.uef.fi)), although the average temperature variations are substantial. The growing season, i.e. average daily temperatures greater than 5 °C, is approximately 155 days ([www.uef.fi](http://www.uef.fi)). The annual precipitation in the area is 700–750 mm and has a yearly snow cover of 165–175 days ([www.fmi.fi/vuositilastot](http://www.fmi.fi/vuositilastot)).

Paukkajanvaara was operating as a pilot U mine between 1958 and 1961 (Äikäs, 2012) after the discovery of relatively high U concentrations in the area in the 1950's. However, mining operations were deemed uneconomic and ceased after producing 30 t of U from 40,000 t of ore grading 0.14 wt% of U (Pohjolainen, 2015). The ore was milled and U enriched on the site during the mining operations. In the late 1970's the entrance to the mining shaft was covered with concrete and the area was abandoned. In the early 1990's an increase in radiation levels by 0.5 µS/h at the Paukkajanvaara site were reported by the Finnish Radiation and Nuclear Safety Authority (STUK). The remediation of the site was initiated in 1992 and over the following two years the mineshaft and pit were demolished and the entire area was covered with a 30 cm thick clay layer, which was further covered by a 1.2 m thick layer of till. Sediments in the ponds and stream in the area were left in place.

### 2.2. Sample collection

A total of 17 samples were collected from the Paukkajanvaara area in June 13th 2017. Of these, 13 soil samples were collected from top organic layer as well as from the top mineral layer (up to 5 cm) from the tailings area/area between tailings and Iso Hiislampi pond and from the waste rock area (Fig. 1, Table 1). In addition, one moss sample, two samples of top layer sediment from Iso Hiislampi pond and one sample from the North end of the ditch running from the waste rock pile area through the tailings area to the Iso Hiislampi pond were





**Fig. 1.** The Paukkajanvaara sampling area. A) Map of the area, B) Iso Hiislampi pond (N = 6981371.990, E = 653303.850), C) Fe dike (N = 6981058.990, E = 653233.850), D) Tailings site/ the quagmire from where the samples were obtained (N = 6981303.990, E = 653239.850), E) Road near the Waste rock pile area (N = 6981121.990, E = 653105.850), F) Fe hill area. The soil samples were taken from the shores between the ditch and the rocky hills (N = 6981183.990, E = 653285.850). All coordinates are presented in ETRS-TM35FIN –system.

collected. From each sampling point two to three parallel samples were collected and one parallel sample was randomly chosen for the following DNA isolations. As the Paukkajanvaara former pilot scale uranium mine area is classified as final disposal site of nuclear waste, complied with the Radiation Act, samples from the lower soil or sediment layers could not be taken, as the protecting soil layers are to be remained intact. Geiger counter was used to detect radiation on each sampling site in-situ. The soil samples were taken aseptically in 50 mL sterile centrifugal tubes, which were sealed with Parafilm around the caps and the tubes were further sealed in plastic. Sediment samples were collected aseptically using sterile 25 mL serological pipettes into 50 mL sterile centrifugal tubes, which were thereafter handled and sealed as described above for the soil samples. All samples were brought to the

laboratory in cooling bags and stored frozen at  $-20^{\circ}\text{C}$ . The samples were thawed immediately before use and used as such.

### 2.3. Nucleic acid isolation

Microbial community DNA was isolated from approximately 0.5 g subsamples of thawed soil, sediment and moss samples with NucleoSpin® Soil DNA extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Germany), using Lysis Buffer SL1 and Enhancer solution SE according to the manufacturer's instructions. Negative DNA isolation controls were included in the isolation protocol. The DNA was eluted in 100  $\mu\text{L}$  elution buffer and the DNA concentration of each extract was measured using the NanoDrop-1000 spectrophotometer (Thermo-Fisher Scientific).

**Table 1**

Paukkajanvaara mine area sample types and sample codes. n.a. refers to no measurement.

| Sampling site          | Sample type                   | Sample code/Sampling layers          | Activity measured on site (cps) | Distance from the Iso Hiislampi pond shore (m) |
|------------------------|-------------------------------|--------------------------------------|---------------------------------|--|
| Mill tailings/quagmire | Moss                          | 8 moss                               | n.a.                            | n.a.   |
| Mill tailings/quagmire | Top organic and mineral layer | 8A organic                           | 100–200                         | 9  |
| Mill tailings/quagmire | Top organic and mineral layer | 8B organic, 8B mineral               | 20                              | 14   |
| Mill tailings/quagmire | Top organic and mineral layer | 8C organic, 8C mineral               | 30                              | 17   |
| Mill tailings/quagmire | Top organic and mineral layer | 8D organic                           | 9                               | 2  |
| Mill tailings/quagmire | Top organic and mineral layer | 8E organic, 8E mineral               | 250                             | 3  |
| Iso Hiislampi pond     | Top sediment                  | Sediment 1A                          | n.a.                            | n.a.   |
| Iso Hiislampi pond     | Top sediment                  | Sediment 2A                          | n.a.                            | n.a.   |
| Fe dike/Ditch          | Top sediment                  | Fe dike sediment                     | n.a.                            | n.a.   |
| Fe dike/Ditch          | Top soil/sand                 | Fe hill mineral 1, Fe hill mineral 2 | 7                               | n.a.   |
| Waste rock area        | Top moraine                   | WRA moraine                          | 20                              | n.a.   |
| Waste rock area        | Top soil                      | WRB topsoil                          | ~20                             | n.a.   |
| Waste rock area        | Top sand                      | WRC sand                             | ~20                             | n.a.   |

## 2.4. Estimation of microbial community size

The size of the microbial community in the different sampling sites was estimated by bacterial and archaeal 16S rRNA gene qPCR as described in [Bomberg et al. \(2016\)](#) using triplicate samples. The fungal community sizes were estimated targeting the fungal 5.8 rRNA gene using a TaqMan approach, using primers 5.8F1 and 5.8R1 and probe 5.8P1 ([Haugland and Vesper, 2002](#)) as described in [Rajala et al. \(2015\)](#) using triplicate samples.

## 2.5. Amplicon library preparation

The amplification libraries for high throughput sequencing with the Ion Torrent PGM platform were prepared by PCR from the DNA samples. Bacterial 16S rRNA genes were targeted with primers Bact\_0341F/Bact\_805R ([Herlemann et al., 2011](#)), flanking the variable region V3-V4 of the 16S rDNA gene, archaeal 16S rRNA genes with primers S-D-Arch-0349-a-S-17/S-D-Arch-0787-a-A-20 ([Klindworth et al., 2013](#)), targeting the V4 region of the gene, and fungal internal transcribed spacer (ITS) gene markers with primer pair ITS1 and ITS2 targeting the fungal ITS1 region ([Gardes and Bruns, 1993](#); [White et al., 1990](#)). PCR amplification was performed in parallel 25  $\mu$ L reactions for every sample containing 1 $\times$  MyTaq<sup>TM</sup> Red Mix (Bioline, London, UK), 20 pmol of each primer, up to 25  $\mu$ L molecular-biology-grade water (Sigma) and 2  $\mu$ L of template. The PCR program consisted of an initial denaturation step at 95 °C for 3 min, 35 cycles for bacteria and fungi and 40 cycles for archaea of 15 s at 95 °C, 15 s at 50 °C, and 15 s at 72 °C. A final elongation step of 30s was performed at 72 °C. The PCR products were verified with agarose gel electrophoresis. Amplicons were sent to Ion Torrent sequencing with PGM equipment (Bioser, Oulu, Finland) and amplicons were purified before sequencing by the staff at Bioser.

## 2.6. Sequence processing and analysis

The sequence reads were analyzed using the Mothur software (v.1.39.5) ([Schloss et al., 2009](#)) to remove adapter, barcode and primer sequences, and to exclude sequences that did not meet the quality criteria (no barcode and primer mismatches, no ambiguous nucleotides, maximum eight nucleotide long homopolymer stretches and minimum length of 250 bp for bacteria and archaea and 200 bp for fungal ITS sequences). A qwindowaverage of 25 and a qwindowsize of 50 were used on the PGM read data in order to remove erroneous reads from the data set. The bacterial and archaeal 16S rRNA sequences were aligned with Mothur to the Silva version 132 reference alignment ([Pruesse et al., 2007](#); [Quast et al., 2013](#)). Chimeric sequence reads were removed with Chimera Uchime in Mothur using the Silva 132 database as template. A phylip distance matrix was built according to the aligned sequences using the dist.seqs command in Mothur with a cutoff value of 0.03. Sequence reads were clustered in to operational taxonomic units (OTU) sharing 97% identity within each OTU. The OTUs were classified against the Silva.seed\_v132 database in Mothur using the wang classification method.

For the fungal ITS sequences a distance matrix was built from the unaligned representative unique sequences using the pairwise.seq command in Mothur. Sequences were grouped into OTUs sharing 97% internal sequence homology. The representative sequences for each OTU were classified using the dynamic version of the UNITE ITS database (version 6) ([Köljalg et al., 2013](#); [Nilsson et al., 2018](#); [UNITE Community, 2017](#)).

For comparing alphadiversity metrics the sequence data sets were rarefied to 1000 sequences for fungal ITS and 2000 sequences for bacterial and archaeal 16S rRNA gene sequences per sample, where applicable. The alphadiversity analyses included the Shannon and Simpson diversity indices and the estimated Chao and ACE OTU richness.

The sequences have been submitted to the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under study accession number PRJEB30939.

## 2.7. Radium concentrations, pH and dry matter content

Gamma spectrometry was used for the estimation of <sup>226</sup>Ra concentrations in the dried and sieved soil, sediment and moss samples. All samples were oven dried at 40 °C to a constant weight and sieved through a 2 mm sieve. Dry weight (DMC, %) was determined from triplicate samples at 105 °C using a Sartorius MA 150-moisture analyzer according to manufacturer's instructions. To prevent the escape of radon from the samples and consequently to ensure the radioactive equilibrium between <sup>226</sup>Ra and <sup>214</sup>Pb, the samples were sealed in vacuum bags for three weeks ([Herranz et al., 2006](#)), after which the samples were measured using a Canberra XTRA gamma counter with Extended Range Coaxial HPGe Detector using calibrated measurement geometries. Radium (<sup>226</sup>Ra) concentration was determined from the emission of the progeny nuclide <sup>214</sup>Pb, which was assumed to be in secular equilibrium with <sup>226</sup>Ra ([Herranz et al., 2006](#)), and the spectra were analyzed using GXRS232 Spectral Analysis Program. pH was determined from duplicate thawed fresh sediment and soil samples in 0.01 M CaCl<sub>2</sub> solution using ISO 10390:2005 standard.

## 2.8. Sulfate concentrations

As radium readily precipitates with sulfate (SO<sub>4</sub><sup>2-</sup>) as RaSO<sub>4</sub> or coprecipitates with barium as (Ba,Ra)SO<sub>4</sub>, sulfate concentrations were determined from duplicate thawed, fresh aliquots of soil, sediment and moss samples. 0.5 g of each sample was weighted into a 50 mL centrifugal tube and 7.5 mL (1:15) of 0.1 M HCl was added ([Liang et al., 2010](#)). The samples were incubated in room temperature for 2 h, after which the samples were centrifuged at 12000g for 10 min and filtered through a 0.2  $\mu$ m membrane filter. Sulfate concentrations, as well as other oxyanions (nitrate (NO<sub>3</sub><sup>-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>)), were determined from filtered samples using IC (Metrohm MIC-12) and validated SFS-EN ISO 10304-1 standard method with corresponding standard solutions. Other anions could not be determined due to broad Cl<sup>-</sup> peak in the IC spectra, caused by HCl. Blank samples were used to detect any possible contamination in all determinations.

## 2.9. Isolation and identification of bacteria

Bacteria were isolated from Paukkajanvaara mine area samples using a serial dilution method and Plate Count Agar (PCA, Standard Methods Agar) (5% Peptone, 2.5% Yeast Extract and 1% Glucose) (BDH Chemicals, pH 7), *Thiobacillus* Medium (pH 6.6) ([https://www.dsmz.de/microorganisms/medium/pdf/DSMZ\\_Medium36.pdf](https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium36.pdf)) and *Acidithiobacillus ferrooxidans* Medium (pH 4.4) ([https://www.dsmz.de/microorganisms/medium/pdf/DSMZ\\_Medium71.pdf](https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium71.pdf)). *Thiobacillus* and *Acidithiobacillus ferrooxidans* media were supplemented with 12 g agar/1 L medium. PCA was used as non-selective, neutral medium. As Paukkajanvaara ore contains iron sulfides like pyrites (FeS<sub>2</sub>) ([Piirainen, 1968](#)), *Thiobacillus* and *Acidithiobacillus ferrooxidans* media were in addition used to isolate e.g. sulfur and iron oxidizing bacteria and to provide lower pH for more acidophilic bacteria. The method previously described by [Lusa et al. \(2016\)](#) was used with the exception of 0.01 M Phosphate buffer (pH 7) being used for dilutions instead of sterile water. The plates were incubated at 20 °C for up to 35 days (840 h) to enable also the growth of more slowly growing boreal bacterial strains. A temperature of 20 °C was chosen to correspond the approximate maximum average surface soil temperature found in Eastern-Finland region in the summer months (June–August) ([Lusa et al., 2015](#); [Lusa et al., 2016](#)). The growth on plates was checked every 24 h (24–840 h) and as bacterial colonies were observed only on the PCA plates, further pure cultures were prepared from isolated colonies on PCA only.



Bacterial cells from each pure culture were Gram stained and examined using a light microscope (Nikon ECLIPSE E200) with 1000-fold magnification. Catalase activity was tested as described previously (Lusa et al., 2016). RapID™ ONE system (Remell), RapID™ NF Plus systems (Remell) and ERIC® electronic code were used according to the manufacturer's instructions for further characterization of the isolated Gram-negative and oxidase negative bacteria.

For the identification of bacterial isolates by sequencing, one colony of bacterial biomass was collected directly from the PCA plate of each isolate using a sterile pipette tip (Lusa et al., 2016). The bacterial mass was suspended in the lysis buffer SL1 and Enhancer solution SE of the NucleoSpin Soil DNA extraction kit (Macherey-Nagel), after which the DNA was extracted according to the manufacturer's instructions. The DNA was subsequently sent for PCR amplification, fragment purification and Sanger sequencing of the 16S rRNA gene to MacroGen Inc., Netherlands, where PCR amplification and sequencing was conducted in both directions using primers 27f, 518f, 800r and 1492r (Lane, 1991). For quality control, the isolated DNA of *Pseudomonas* spp. strains PS-0-L and T5-6-I were sequenced from duplicate samples in two different occasions to ensure the validity of the sequences.

Sequence chromatograms were imported into Geneious Pro v. 10.1.3 in abi1 format (Biomatters Ltd., Auckland, New Zealand) and processed for phylogenetic analyses. The sequences were assembled and edited by hand. In order to identify the isolated bacteria, the obtained 16S rRNA gene sequences were compared to the sequences deposited in GenBank using the blastn tool in Geneious Pro. The closest matching sequences as well as relevant reference sequences were aligned using the MUSCLE (Edgar, 2004) alignment tool in Geneious Pro. A maximum likelihood phylogenetic tree was calculated from the alignments using the PhyML tool and the Jukes-Cantor (Jukes and Cantor, 1969) substitution model, and the topology of the tree was tested by bootstrap analyses of 1000 random resamplings. The sequences have been deposited in GenBank under accession numbers MK281650–MK281651.

#### 2.10. Uptake experiments of $^{226}\text{Ra}$ with *Pseudomonas* spp. strains

Of the isolated bacterial strains, the two *Pseudomonas* spp. strains, P5 and P20, were chosen for the further uptake experiments with  $^{226}\text{Ra}$ , based on the dominance of *Pseudomonas* spp. in the  $\gamma$ -proteobacterial community in the Paukkajanvaara area (see below), and the strong growth of these isolates, suitable for in-vitro uptake experiments. In addition, two *Pseudomonas* spp. strains PS-0-L and T5-6-I previously isolated from Lastensuo bog on PCA plates (Lusa et al., 2016) were used in the uptake experiments as reference strains. Bacteria were cultured aerobically on sterile PCA growth plates at 20 °C in the dark (Lusa et al., 2016). The colonies were transferred onto new plates weekly. A batch equilibrium method was used to determine the uptake of  $^{226}\text{Ra}$  by the isolated bacteria as described previously in Lusa et al., 2016. The uptake experiments were carried out using 100 Bq of  $^{226}\text{Ra}(\text{NO}_3)_2$  in two different liquid media (A and B) using three incubation temperatures (4 °C, 20 °C and 37 °C) with triplicate samples for each medium (Lusa et al., 2016). The  $^{226}\text{Ra}$  activity of 100 Bq was chosen based on the minimum detectable activity (MDA) of 2 Bq and maximum of 98% biosorption capacity of  $^{226}\text{Ra}$ . Medium A consisted of 1% Tryptone and 0.5% NaCl (pH 7), medium B of 1% yeast extract and 0.5% NaCl (pH 7) (Lusa et al., 2016). These media and pH 7 were chosen for the uptake experiments as the effects of the two main components (Tryptone ~ Peptone) of the PCA (pH 7) medium on which all *Pseudomonas* spp. strains selected for these experiments were originally isolated on, on  $^{226}\text{Ra}$  uptake was to be tested. Bacterial suspensions of the *Pseudomonas* spp. strains P5, P20, PS-0-L and T5-6-I were prepared for pre-incubation by transferring one loop of stationary phase bacterial colonies from the PCA growth plates into 80 mL of medium A or B. As all used *Pseudomonas* spp. strains were found to reach the plateau phase within 24–48 h, the suspensions were incubated for 24–48 h on an orbital shaker (120 rpm) at 20 °C in order to allow the cultures to reach the plateau

phase. After the incubation, the absorbance of the bacterial cultures was recorded at 660 nm and 5.1 mg (DW) of each bacterium was used in the following uptake experiments. Batch experiments were performed using triplicate samples in 15 mL sterile centrifuge tubes in which 5 mL of medium A or B, 5.1 mg (DW) of bacteria and 100  $\mu\text{L}$  of  $^{226}\text{Ra}(\text{NO}_3)_2$  solution corresponding to an activity of 100 Bq were added. In addition, the effect of pH between pH 6 and pH 8 was tested using *Pseudomonas* sp. P5 and 1% Tryptone broth by adjusting the pH of the solution between pH 6 and 8 using 1 M HCl or 1 M NaOH depending on the desired pH (Lusa et al., 2016). All suspensions were incubated for 1–3 days at 4 °C, 20 °C or 37 °C in the dark, after which the suspensions were filtered through a 0.2  $\mu\text{m}$  pore-size sterile membrane filters (PALL Life Sciences, Acrodisc®, Supor®) (Lusa et al., 2016; Lusa et al., 2017). The resulting solution was used for the gamma spectrometric (Hidex Automatic Gamma Counter) determination of  $^{226}\text{Ra}$  activity in the solution using a 186 keV gamma peak. In addition, standard solutions and suspensions without added bacteria (blank samples) were prepared accordingly and measured to assure that no sorption of radium on laboratory equipment, filters or nutrient broth solutions occurred (Lusa et al., 2016). The association of  $^{226}\text{Ra}$  on bacterial cells was calculated from the difference between initial and final (after filtration)  $^{226}\text{Ra}$  concentration in the solution and expressed as correlation coefficient:  $K_d = [(A_i - A_f) / A_f] \times (V/m)$ , where  $A_i$  (Bq/L) and  $A_f$  (Bq/L) are the initial  $^{226}\text{Ra}$  activity and final activity concentration of the solution,  $V$  (L) is the solution volume, and  $m$  (kg) is the sample mass at  $t = 0$  (Lusa et al., 2016).

#### 2.11. The effect of *Pseudomonas* spp. strains on $^{226}\text{Ra}$ extraction from soil samples

In addition to uptake experiments, *Pseudomonas* spp. strains were used to study their effect on the leaching of  $^{226}\text{Ra}$  from the Paukkajanvaara area soil samples. To study the difference between pristine population, *Pseudomonas* spp. strains and sterile sample, 2 g aliquots of 8E mineral layer samples were sterilized by autoclaving three times at 125 °C/1 bar over pressure for 20 min (Lusa et al., 2015). The second and third autoclaving was performed 7 and 10 days after the initial autoclaving. After sterilization 15 mL of medium A and 5.1 mg (DW) of each bacterial strain was added, after which the samples were incubated for 7 or 14 days at 20 °C on a rotary shaker (13 rpm). After incubation, the samples were filtered through a sterile 0.45  $\mu\text{m}$  pore-size membrane filter (PALL Life Sciences, Acrodisc®, Supor®) in order to also remove the  $^{226}\text{Ra}$  possibly associated with the aquatic suspended bacteria. Thus, only the tightly bound Ra associated with the mineral fraction of the soil sample or with the biofilms attached to the mineral fraction remained on the solid phase. After filtration, the samples were sealed in vacuum bags for three weeks to prevent the escape of radon from the samples and consequently to ensure the radioactive equilibrium between  $^{226}\text{Ra}$  and  $^{214}\text{Bi}$  (bismuth) (Herranz et al., 2006), which after the samples were measured using a Canberra XTRA gamma counter as described in Section 2.7. Aliquots of the solutions were used for pH and sulfate concentration measurements from duplicate samples. pH was measured from the aliquot solutions using Thermo Scientific Orion Star A111 pH meter and sulfate concentrations were measured as described in Section 2.8.

#### 2.12. Statistical analyses

The relationships between the measured  $^{226}\text{Ra}$  and main anion concentrations, bacterial and archaeal 16S rRNA and fungal 5.8 rRNA gene copies, community richness (Chao 1) and diversity (Shannon index  $H'$ ) of the bacterial, archaeal and fungal communities were analyzed by univariate analysis. Prior to the analysis, the normal distribution of the data was evaluated by the Shapiro-Wilk test ( $p < 0.05$ ). The normality hypotheses were rejected for bacterial, archaeal 16S rRNA gene copies and fungal 5.8S rRNA gene copies as

well as for  $^{226}\text{Ra}$  concentrations. Dry matter (%), diversity index (Shannon), richness (Chao1) and number of observed OTUs (sobs) were close to normal distribution. Therefore, Spearman's correlation analysis was used, since this correlation coefficient does not assume linear relationship among variables. All statistical analyses were performed using Origin-Pro 2018 (OriginLab, USA).

The similarity between the microbial community profiles of the different samples was analyzed with Phyloseq (McMurdie and Holmes, 2013) in R (R Core Team, 2014). Principal Coordinates analyses (PCoA) were performed on all samples with >100 sequence reads using the Bray–Curtis distance model. Environmental parameters having a statistically significant ( $p < 0.05$ , 999 permutations) impact on the distribution of the samples on the PCoA plots were plotted as vectors on the plots.

To study the statistical difference between the number of bacterial and archaeal 16S rRNA and the fungal 5.8S rRNA gene copies  $\text{g}^{-1}\text{DW}$ , the effect of different growth conditions on radium uptake (i.e. the difference between nutrient broths A and B and the temperatures 4 °C, 20 °C and 37 °C) and the effect of different bacterial strains on the amount of leached radium and sulfate, the analysis of variance was performed using OriginPro 8.6 (OriginLab®) and one-way ANOVA at the  $p < 0.05$  level. Prior to analysis, Shapiro–Wilk test ( $p < 0.05$ ) was used to examine the normality of the data. The normality hypotheses were rejected for bacterial and archaeal 16S rRNA and fungal 5.8S rRNA gene copies as well as for temperature, nutrient broths and  $^{226}\text{Ra}$  uptake in bacterial strains. Therefore, logtransformed data was used in one-way ANOVA in these tests. Analysis of variance was done for all studied bacteria separately for temperature, nutrient broth, sulfate leaching and  $^{226}\text{Ra}$  leaching.

### 3. Results

#### 3.1. Radium and major anion concentrations, pH and dry matter content

Dry matter content (%) of all organic soil samples (8A–8E organic layers) varied between 17 and 35%, with an average of 24% (Table 2). Higher dry matter content was observed in the mineral soil samples (8B–E mineral layer, Fe hill mineral and WRA–WRC top soil) with an average dry matter content of 70%. Highest dry matter content of 90% was found in the WRA moraine sample.

Nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ) and sulfate ( $\text{SO}_4^{2-}$ ) concentrations varied considerably, depending on sampling site (Table 2). The highest  $\text{NO}_3^-$  concentration of 1145 mg/kg DW was observed in the 8 moss sample. In the majority of the samples the  $\text{NO}_3^-$  concentrations were,

however, low and largely below the detection limit (MDL 0.47 ppm) (Table 2). In accordance to the  $\text{NO}_3^-$  concentrations, also  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  concentrations varied substantially between the samples and in part of the samples the concentrations were below the detections limits (0.07 ppm and 0.14 ppm for  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ , respectively) (Table 2). The highest  $\text{PO}_4^{3-}$  concentration, 360 mg/kg DW, was detected in the 8A organic sample and the highest  $\text{SO}_4^{2-}$  concentrations of 309 mg/kg DW and 307 mg/kg DW were observed in the 8 moss and 8A organic layer samples, respectively.

The highest  $^{226}\text{Ra}$  concentrations were detected in the mill tailings area near the Iso Hiislampi pond. In the organic and mineral soil samples (8A–8E) from this area the highest  $^{226}\text{Ra}$  concentration of 21 Bq  $\text{g}^{-1}\text{DW}$  was observed in the 8E organic layer sample. A high  $^{226}\text{Ra}$  concentration of 16 Bq  $\text{g}^{-1}\text{DW}$  was also detected in the mineral layer of this same sampling point.

Only minor variation in the pH values of the soil and sediment samples of the mine area were observed (Table 2.). In the soil samples retrieved from the mill tailings area the average pH value was 4, with a range from 3.6 to 4.8. Slightly higher average pH, 4.5, was observed in the waste rock pile area samples. Highest pH of 5.1 was found in the Iso Hiislampi pond sediment sample (8 sediment 1A).

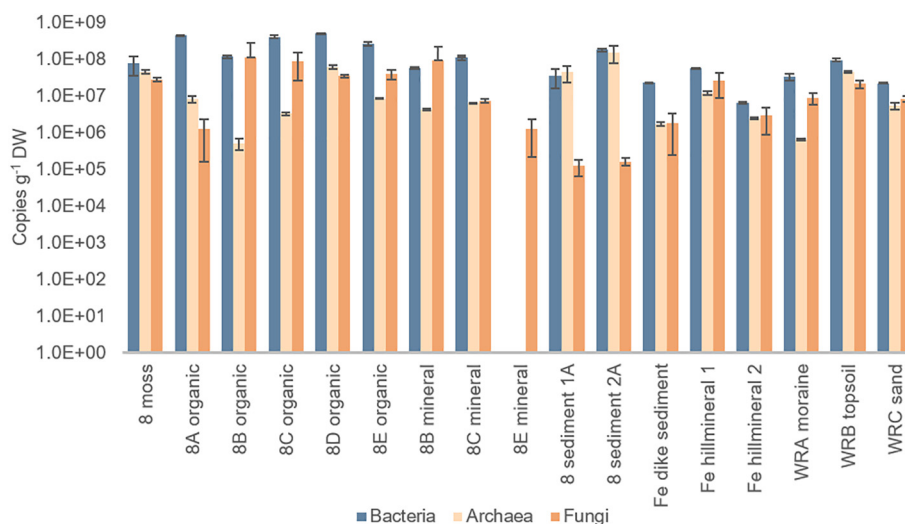
#### 3.2. The size of bacterial, archaeal and fungal communities

Bacteria were more prevalent in most of the samples, compared to archaea and fungi (Fig. 2). The numbers of bacterial 16S rRNA gene copies varied between  $6 \times 10^6$ – $5 \times 10^8$  copies  $\text{g}^{-1}\text{DW}$ , with higher numbers found in the mill tailings area samples (on average  $2 \times 10^8$  copies  $\text{g}^{-1}\text{DW}$ ), compared to the waste rock pile area (on average  $5 \times 10^7$  copies  $\text{g}^{-1}\text{DW}$ ) and sediment and Fe hill samples (on average  $8 \times 10^7$  copies  $\text{g}^{-1}\text{DW}$  and  $3 \times 10^7$  copies  $\text{g}^{-1}\text{DW}$ , respectively). The number of archaeal gene copies ranged from  $5 \times 10^5$  copies  $\text{g}^{-1}\text{DW}$  to  $1 \times 10^8$  copies  $\text{g}^{-1}\text{DW}$  in the samples. Highest archaeal 16S rRNA gene copy numbers were found in the sediment samples (on average  $6 \times 10^7$  copies  $\text{g}^{-1}\text{DW}$ ). Similar numbers for archaeal gene copies were found in the mill tailings and waste rock pile area samples (on average  $2 \times 10^7$  copies  $\text{g}^{-1}\text{DW}$  in both areas). Considerably lower, on average  $7 \times 10^6$  copies  $\text{g}^{-1}\text{DW}$ , were obtained for Fe hill samples. However, for sample 8E mineral the bacterial and archaeal signal in the qPCR assay remained below the detection limit of the assay. Fungal 5.8S rRNA gene copies were obtained from all samples. The number of 5.8S rRNA gene copies varied between  $1 \times 10^5$  and  $1 \times 10^8$  copies  $\text{g}^{-1}\text{DW}$ . Highest numbers of fungal 5.8S rRNA gene copies were obtained for 8B and 8C organic ( $1 \times 10^8$  and 8

**Table 2**  
The concentration of  $^{226}\text{Ra}$  (Bq  $\text{g}^{-1}\text{DW}$ ), the dry matter content (%) and concentrations of  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  (mg  $\text{kg}^{-1}\text{DW}$ ) in Paukkajanvaara moss, soil and sediment samples. < indicates measurements below detection limit of the assay<sup>a</sup>, n.a. indicates that the analyses were not done. DMC = Dry matter content. All coordinates are presented in ETRS-TM35FIN –system.

| Sample            | Sampling area coordinates       | Ra-226 (Bq $\text{g}^{-1}$ ) | DMC (%) | pH   | $\text{NO}_3^-$ (mg $\text{kg}^{-1}$ ) | $\text{PO}_4^{3-}$ (mg $\text{kg}^{-1}$ ) | $\text{SO}_4^{2-}$ (mg $\text{kg}^{-1}$ ) |
|-------------------|---------------------------------|------------------------------|---------|------|--|---|---|
| 8 moss            | N = 6981303.990, E = 653239.850 | 2.8                          | 15      | 4.2  | 1145                                   | 97  | 309                                       |
| 8A organic        | N = 6981303.990, E = 653239.850 | 11                           | 17      | 3.6  | <                                      | 360                                       | 307                                       |
| 8B organic        | N = 6981303.990, E = 653239.850 | 3.4                          | 35      | 3.9  | <                                      | <   | 121                                       |
| 8C organic        | N = 6981303.990, E = 653239.850 | 2.7                          | 35      | 3.7  | <                                      | 106                                       | <   |
| 8D organic        | N = 6981303.990, E = 653239.850 | 1.4                          | 16      | 3.8  | <                                      | 80  | 171                                       |
| 8E organic        | N = 6981303.990, E = 653239.850 | 21                           | 19      | 4.2  | <                                      | <   | <   |
| 8B mineral        | N = 6981303.990, E = 653239.850 | 4.0                          | 78      | 4.1  | 36                                     | 21  | 67  |
| 8C mineral        | N = 6981303.990, E = 653239.850 | 0.1                          | 71      | 3.8  | <                                      | 112                                       | <   |
| 8E mineral        | N = 6981303.990, E = 653239.850 | 16                           | 44      | 4.3  | <                                      | 56  | <   |
| 8 sediment 1A     | N = 6981371.990, E = 653303.850 | 1.6                          | 43      | 5.1  | 3                                      | 102                                       | 33  |
| 8 sediment 2A     | N = 6981371.990, E = 653303.850 | n.a.                         | n.a.    | n.a. | <                                      | <   | <   |
| Fe dike sediment  | N = 6981058.990, E = 653233.850 | 4.8                          | 88      | 4.2  | <                                      | 185                                       | <   |
| Fe hill mineral1  | N = 6981183.990, E = 653285.850 | 0.0                          | 42      | 4.4  | 48                                     | <   | <   |
| Fe hill mineral 2 | N = 6981183.990, E = 653285.850 | 0.1                          | 79      | 4.3  | <                                      | <   | <   |
| WRA moraine       | N = 6981121.990, E = 653105.850 | 0.1                          | 90      | 4.4  | <                                      | 178                                       | <   |
| WRB topsoil       | N = 6981121.990, E = 653105.850 | 0.02                         | 69      | 4.2  | 5                                      | <   | <   |
| WRC sand          | N = 6981121.990, E = 653105.850 | 0.05                         | 89      | 4.8  | 2                                      | <   | <   |

<sup>a</sup> Detection limits  $\text{NO}_3^- = 0.47$  ppm,  $\text{PO}_4^{3-} = 0.07$  ppm,  $\text{SO}_4^{2-} = 0.14$  ppm.



**Fig. 2.** Number of bacterial and archaeal 16S rRNA and Fungal 5.8S rRNA gene copies g<sup>-1</sup> DW in the Paukkajanvaara area samples. Based on *t*-test the means of the numbers of log transformed gene copies of bacterial and archaeal 16S rRNA and Fungal 5.8S rRNA gene copies g<sup>-1</sup> DW did not statistically differ from each other.

$\times 10^7$  copies g<sup>-1</sup> DW, respectively), and 8B mineral samples ( $9 \times 10^7$  copies g<sup>-1</sup> DW). Highest average numbers of fungal 5.8S rRNA gene copies were found in the mill tailings area ( $4 \times 10^7$  copies g<sup>-1</sup> DW). The waste rock pile area and Fe hill area samples had similar average numbers of fungal 5.8S rRNA gene copies ( $1 \times 10^7$  copies g<sup>-1</sup> DW). The lowest numbers of fungal 5.8S rRNA gene copies were obtained for the sediment samples, on average  $7 \times 10^5$  copies g<sup>-1</sup> DW. The numbers of log transformed bacterial and archaeal 16S rRNA and fungal 5.8S rRNA gene copies g<sup>-1</sup> DW did not statistically differ from each other using one-way ANOVA.

### 3.3. Bacterial, archaeal and fungal diversity

Amplicon sequencing of bacterial and archaeal 16S rRNA genes identified a total of 31,585 and 2591 bacterial and archaeal Operational Taxonomic Units (OTUs), respectively, when clustered at 97% sequence homology. For fungal ITS sequences a total of 3233 OTUs were identified.

In order to compare the alphadiversity between samples the data was randomly subsampled (rarefied) to 2000 sequence reads for the bacterial communities, where possible. The highest number of bacterial OTUs in the rarefied data was observed in the 8 moss sample (1710 identified and up to 13,049 estimated (Chao 1) bacterial OTUs (Appendix 1, Table A1)). This sample also had the highest diversity index ( $H' = 7.3$ , rarefied to 2000 reads/sample). The lowest number of identified (490) and Chao1 estimated OTUs (1762) and diversity index ( $H' = 4.70$ ) were found in the 8C organic sample. For all organic layer samples (8A–8E) the number of identified sequences were below 1800 sequence reads per sample and the observed and estimated bacterial OTUs for non-rarefied data were below 1050 and 4200, respectively (Appendix 1, Table A1).

The highest number of archaeal OTUs in the rarefied (1000 randomly subsampled reads) data was observed in the 8 sediment 1A sample with 360 identified and up to 1067 estimated (Chao 1) archaeal OTUs (Appendix 1, Table A2). This sample had also the highest archaeal diversity index ( $H' = 5.0$ , rarefied to 1000 sequence reads/sample). The lowest number of identified (1) and estimated OTUs (1) and diversity index ( $H' = 0$ ) was detected in the Fe hill mineral sample 1. In the Fe hill mineral sample 2 a clearly higher number of observed (72) and estimated (127) OTUs were recorded. For all organic layer samples from the tailings area (8A–8E) the number of identified sequences were below 400 and the observed and estimated archaeal OTUs for non-normalized data were below 90 and 180, respectively (Appendix 1,

Table A2). Lowest number of observed OTUs (20) and estimated number of OTUs (43) for non-normalized archaeal data was found in the 8B organic layer sample.

Based on the rarefied (1000 randomly subsampled sequence reads) fungal sequence data, the highest number of fungal ITS OTUs was observed in the 8 sediment 1A sample from the Iso Hiislampi pond near the tailings area (Appendix 1, Table A3). In this sample, 322 identified and up to 777 estimated (Chao 1) fungal OTUs were observed. This sample also had the highest fungal diversity index ( $H' = 4.8$ ). Equally high diversity index ( $H' = 4.8$ ) was found in the 8C mineral samples taken near Iso Hiislampi pond. In this sample, 306 identified and 776 estimated OTUs were found. The lowest number of identified (105) and estimated fungal OTUs (280) and diversity index ( $H' = 2.2$ ) for the rarefied data was detected in the WRB topsoil sample. For 8B, 8C and 8E organic layer samples from the tailings area the number of identified fungal sequences was <300 in all samples. In the 8E organic layer sample, no fungal sequences were identified. The estimated fungal OTUs in 8B and 8C samples for non-normalized data were 115 and 9 for 8B and 8C, respectively (Appendix 1, Table A3).

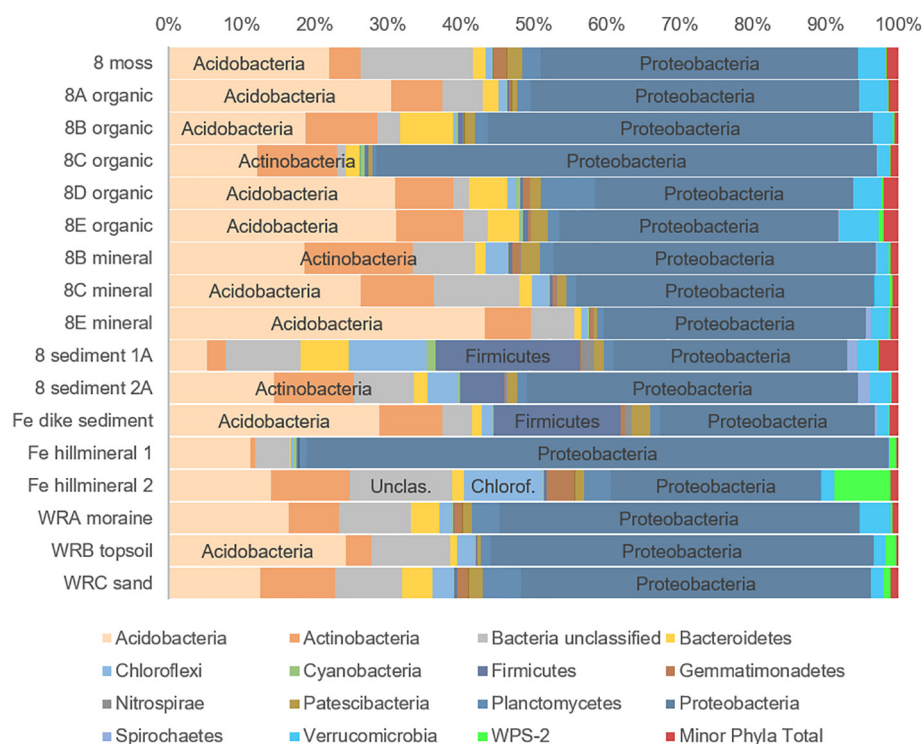
### 3.4. Bacterial, archaeal and fungal community compositions

A total of 46 different bacterial phyla, consisting of 14 major phyla with relative abundance of >1% of all bacterial sequence reads at least in one of the samples, were detected in Paukkajanvaara mine area (Fig. 3, Appendix 1, Fig. A1). The phyla were represented by 814 bacterial genera. Most of these genera were, however, present only at very low relative abundances, <0.001%, and only 80 genera contributed with >1% of the sequence reads in at least one sample (Appendix 1, Fig. A2).

The bacterial community in the area was dominated by *Proteobacteria* and acidophilic *Acidobacteria*. Of these two phyla, *Proteobacteria* covered 29%–80% of all sequence reads of each sample (Fig. 3). The lowest proteobacterial relative abundance was found in the Fe dike sediment sample (29%) and highest in the Fe hill mineral 1 sample (80%). *Acidobacteria* were common (20% of all bacterial reads) in all samples from the mill tailings area (Table 1) and 5%–43% of all bacterial 16S rRNA gene sequence reads of each sample, were covered by *Acidobacteria* (*Acidobacteriaceae*) (Fig. 3).

The *Actinobacteria* contributed with 6.6% (GM) of the bacterial sequence reads and were found in all samples, with the highest relative abundance of 15% in the 8B mineral sample (Fig. 3). In addition, *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi*, *Bacteroidetes*, *Firmicutes*





**Fig. 3.** Relative distribution of major bacterial phyla in samples collected from Paukkajanvaara mine area. The group 'Minor phyla' contain the combined relative abundance of phyla contributing with <1% of the bacterial community in any sample.

and *Cyanobacteria* were detected in all Paukkajanvaara area samples. Their mean relative abundances were, however, lower and varied between 0.2%–2.2% (GM).

*Acidobacteria* subgroup 2 contributed with 1.6%–13.5% of the total bacterial community in the Paukkajanvaara samples (Appendix 1, Fig. A3 A) and *Proteobacteria* were primarily represented by  $\alpha$ - and  $\gamma$ -proteobacterial lineages, covering 18% and 15% (GM), respectively, of all bacterial reads in the mine area (Appendix 1, Fig. A3 B, Appendix 1, Fig. A4).  $\gamma$ -*Proteobacteria* were clearly dominant in the 8 sediment 1A and 2A samples taken from the Iso Hiislampi pond, representing 20% and 37% of the bacterial communities in these samples, respectively. In addition, in three out of the five organic layer samples taken from the mill tailings area, the bacterial communities consisted mostly (28–54%) of  $\gamma$ -*Proteobacteria* (Appendix 1, Fig. A3 B). The largest groups in the  $\gamma$ -*Proteobacteria* were *Betaproteobacteriales* and *Pseudomonadaceae* (15% and 9%, respectively (Appendix 1, Fig. A4)). *Betaproteobacteriales* were primarily presented by *Burkholderia* (*Burkholderia*-*Caballeronia*-*Paraburkholderia*) and *Pseudomonadaceae* by *Pseudomonas*. *Pseudomonas* were the most common bacteria in the sediment samples (8 Sediment 1A and 2A, and Fe dike sediment) and in the 8C organic sample, covering 9–31% of the bacterial community in these samples. In the 8A and 8B organic, and WRB topsoil samples, *Burkholderia* dominated the community and contributed with 14–20% of the bacterial sequence reads.

$\alpha$ -*Proteobacteria* were especially prominent in the 8 moss sample (31% of all bacterial sequence reads), both Fe hill mineral samples (77% and 21% of bacterial reads) and waste rock pile area samples (on average 27% of bacterial reads) (Appendix 1, Fig. A3 B).  $\delta$ -*Proteobacteria* were found only in low abundances and constituted on average < 0.2% of the bacterial reads in all samples (Appendix 1, Fig. A4).

*Actinobacteria* covered on average 8% of the bacterial reads and were represented by unclassified *Acidimicrobiia*, *Acidimicrobiia* IMCC26256, uncultured *Acidimicrobiia*, *Mycobacterium*, unclassified *Microbacteriaceae*, unclassified *Micrococcaceae* and *Streptacidiphilus* (Appendix 1, Fig. A3 C). In addition, 76 minor actinobacterial genera,

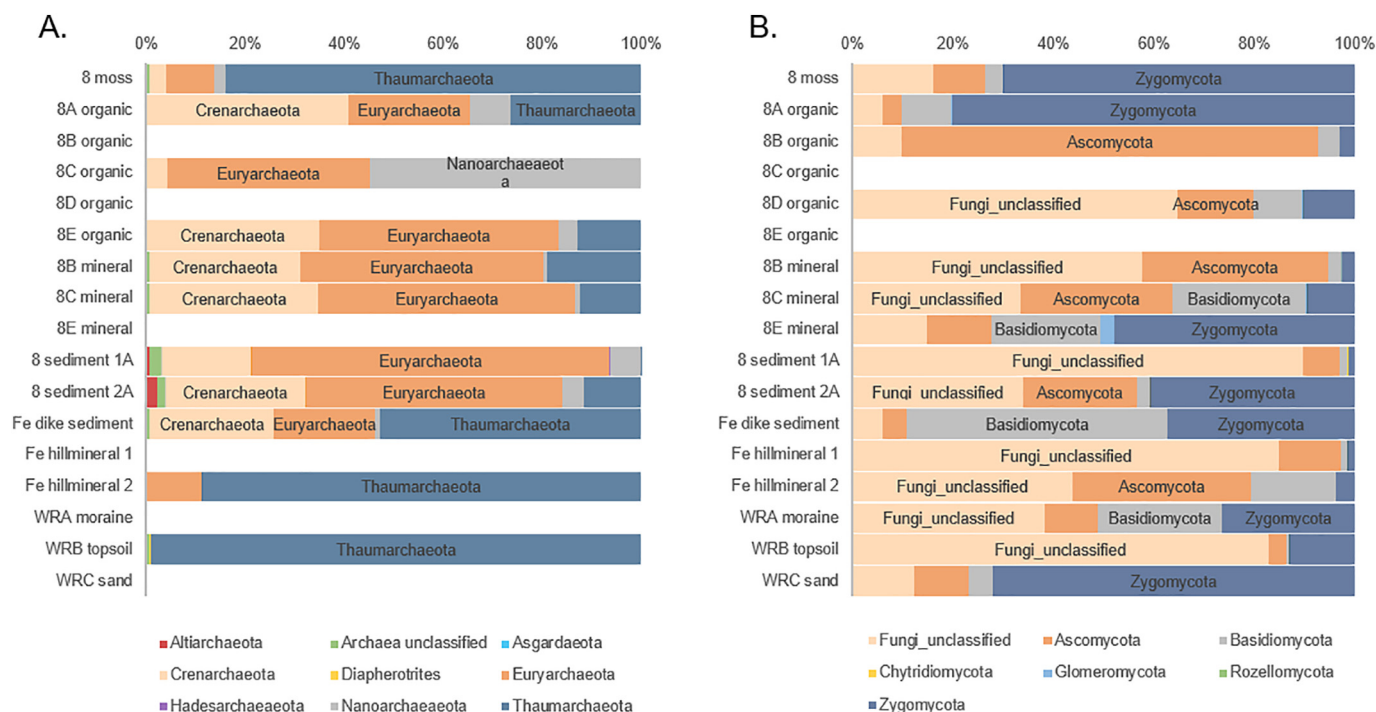
constituting in total < 6% of the bacterial reads in each sample, were detected in the mine area samples, of which *Thermoleophilum* and unclassified *Actinobacteria* were the most common ones. A reasonably high relative abundance of bacteria belonging to unclassified *Acidimicrobiia* (8.1%) were found in the 8 sediment 2A sample. In addition, unclassified *Micrococcaceae* were present in corresponding abundance (8.9%) in the 8B mineral sample.

*Firmicutes* were represented by five main genera, *Tumebacillus*, *Paenibacillus*, unclassified *Clostridiaceae*, *Clostridium sensu stricto* 1 and *Clostridium sensu stricto* 13, but were detected only at low relative abundances (<0.5%) in all other, except for the sediment samples in which *Firmicutes* constituted a substantial portion (6%–20%) of the bacterial community (Appendix 1, Fig. A3 D).

*Spirochaetes* were found in part of the samples with mean relative abundances below 0.3%. Sequences that could not be assigned to any specific bacterial lineage contributed with an average of 6.2% (GM) of the sequence reads with the highest relative abundance of 15% in the 8 moss sample.

The archaeal community in the Paukkajanvaara mine area, consisted of a total of nine archaeal phyla, represented by 54 different genera. The majority belonged to the *Euryarchaeota*, *Nanoarchaeota* and the TACK superphylum archaea *Crenarchaeota* and *Thaumarchaeota* (Fig. 4A). Of these, *Euryarchaeota* (10%–72%) and *Crenarchaeota* (3%–35%) dominated the archaeal community, especially in the samples taken from the mill tailings area and Iso Hiislampi pond (Fig. 4A). *Thaumarchaeota* were especially abundant in 8 moss (84% of archaeal reads), Fe dike sediment (53%), Fe hill mineral 2 (89%) and WRB topsoil samples (99%). *Nanoarchaeota* were most common in 8C organic sample, covering 55% of the archaeal reads in this sample. However, in all other samples, *Nanoarchaeota* represented <9% of the archaeal sequences. In addition, *Altitharchaeota*, *Asgardaeota*, *Diapherotrites* and *Hadesarchaeaeota* were present in the Paukkajanvaara area samples. The abundances for these phyla were however low, on average below 0.1% in all samples (Fig. 4A).

Of the 54 genera representing the archaeal community in the Paukkajanvaara area, 18 genera were only found in the sediment



**Fig. 4.** A) Relative distribution of archaeal phyla in samples collected from Paukkajanvaara mine area. The phyla for samples with >100 archaeal sequences are shown. B) Relative distribution of fungal phyla in samples collected from Paukkajanvaara mine area.

samples. Especially the 8 Sediment 1A sample, taken from the Iso Hiislampi pond, had a high diversity of 46 archaeal genera (Appendix 1, Fig. A5). The genus with highest relative abundance (35% of the archaeal community) in this sample was *Methanosaeta*. Archaea belonging to the order *Methanosarcinales* (class *Methanomicrobia*), represented by *Methanosarcina*, *Methanosaeta*, *Candidatus Methanoperedens*, and unclassified *Methanosarcinales*, were found in all mill tailings area samples from where archaeal sequence reads were obtained and accounted for 4%–36% of the archaeal community in these samples (Appendix 1, Fig. A5). *Methanosarcinales* were found also in the other two sediment samples, 8 sediment 2A and Fe dike sediment, representing 18% and 15% of the archaeal reads in these samples, respectively. In addition, a substantial portion of *Methanosarcinales* was recorded in 8C and 8E organic, and 8B and 8C mineral samples. In these samples *Methanosarcinales* covered 24%, 14%, 33% and 14% of the archaeal reads, respectively. In addition to *Methanosarcinales*, uncultured *Methanomassiliicoccaceae*, *Woesearchaeia* and *Candidatus Nitrosotalea* were found in the mill tailings area soil samples (Appendix 1, Fig. A5), with average relative abundances of 4%, 10% and 17%, respectively.

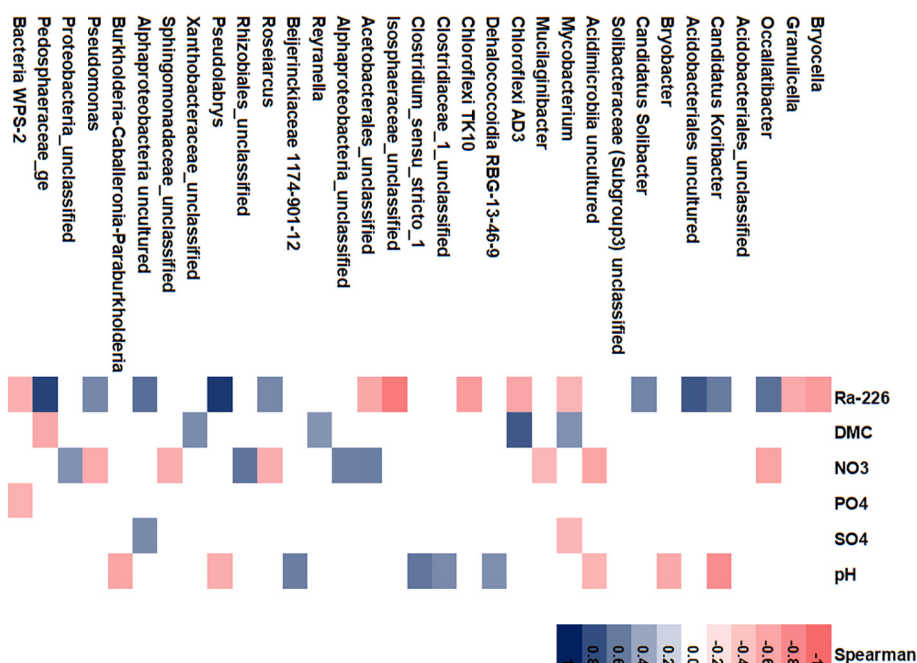
Seven fungal phyla (Fig. 4B), representing 167 different genera (Appendix 1, Fig. A6), were identified in the Paukkajanvaara mine area samples. Fungal ITS sequences were obtained from all samples, except 8E mineral and 8C organic, from which no or only few sequence reads were obtained. *Zygomycota*, *Basidiomycota* and *Ascomycota* were found in all samples from which fungal sequences were obtained. *Zygomycota* dominated especially 8 moss, 8A organic and WRC sand samples, with relative abundances of 70%, 80% and 72% of fungal communities in these samples (Fig. 4B). *Basidiomycota* were most prominent in the 8C organic sample, representing 96% of the fungal community. *Ascomycota* formed a substantial portion of the fungal population in the 8B organic sample, representing 83% of the fungal reads. Fungal OTUs for which the taxonomical affiliation could not be resolved, i.e. Unclassified fungi, were detected in all mine area samples and were especially common in 8D organic (65%), 8E mineral (58%), 8 Sediment 1A (90%), Fe hill mineral 1 (85%) and WRB topsoil (83%) (Fig. 4B). Of the detected genera, 37 genera formed the majority of the fungal

community and 130 genera represented <1% of the sequence reads in any sample (Appendix 1, Fig. A6). Of the major fungal genera, the highest relative abundance in the mine area samples was represented by *Mortierella* (Appendix 1, Fig. A6). This genus contributed with 1.2–80% of the fungal community. *Aspergillus*, *Wilcoxina*, *Ascomycota*, Unclassified *Tremellomycetes*, *Cryptococcus* and *Kabatiella* were also found in high relative abundances (16%–67%) in part of the samples (Appendix 1, Fig. A6). All other fungal genera represented <15% of the fungal community in any sample (Appendix 1, Fig. A6).

### 3.5. Correlation between microbial communities, alphas diversity and physicochemical parameters

Several correlations between bacterial sequence reads and the  $^{226}\text{Ra}$  activities in the area were found. *Candidatus Solibacter*, uncultured *Acidobacteriales*, *Pseudolabrys*, uncultured *Alphaproteobacteria*, and *Pedospaeraceae* had positive correlation with  $^{226}\text{Ra}$  activities ( $r > 0.5$ ,  $p < 0.05$ ) (Fig. 5). Strongest positive correlation with  $^{226}\text{Ra}$  was found for *Pseudolabrys* ( $r = 0.894$ ,  $p < 0.05$ ). In contrast, *Mycobacterium*, *Chloroflexi* AD3 and unclassified *Isosphaeraceae* correlated negatively with  $^{226}\text{Ra}$  activities, the strongest negative correlation ( $r = -0.886$ ,  $p < 0.05$ ) recorded for *Isosphaeraceae* (unclassified). In addition, *Bryocella*, *Granulicella*, *Chloroflexi* TK10, unclassified *Acetobacteriales* and *Bacteria* group WPS-2 all correlated negatively with  $^{226}\text{Ra}$  ( $r < -0.51$ ,  $p < 0.05$ ) and *Occallatibacter*, *Candidatus Koribacter*, *Roseiarcus*, uncultured *Alphaproteobacteria*, and *Pseudomonas* positively ( $r < 0.54$ ,  $p < 0.05$ ). Strong positive correlations with  $^{226}\text{Ra}$  ( $r > 0.65$ ) were however recorded only for *Occallatibacter* and uncultured *Alphaproteobacteria*. Strong negative correlation ( $r < -0.6$ ) with  $^{226}\text{Ra}$  was recorded for both *Bryocella* and *Chloroflexi* TK10. In addition, several correlations between bacterial genera and pH, nitrate, sulfate, phosphate and dry matter content were found (Fig. 5).

The number of bacterial 16S rRNA gene copies correlated negatively ( $r = -0.732$ ,  $p = 0.0008$ ) with the dry matter content (%) and pH ( $r = -0.793$ ,  $p = 0.0002$ ). No correlation between the number of bacterial 16S rRNA gene copies, bacterial community richness (Chao1) and



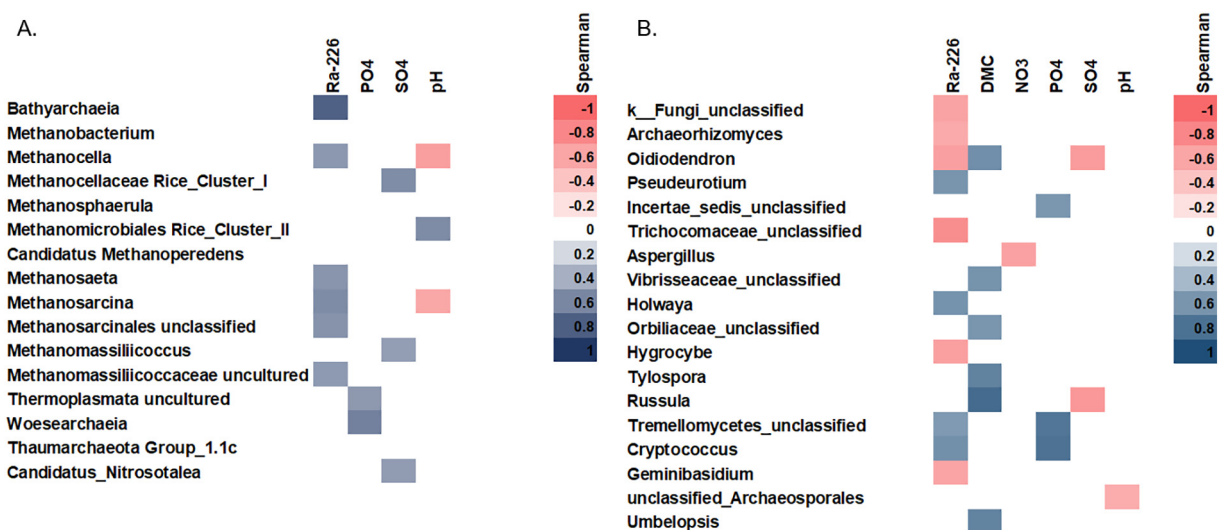
**Fig. 5.** Heat map showing Spearman correlation coefficients ( $p < 0.05$ ) between major bacterial genera in the Paukkajänvaara area and  $^{226}\text{Ra}$  (Bq g<sup>-1</sup> DW), concentrations of major anions (NO<sub>3</sub>, SO<sub>4</sub>, PO<sub>4</sub>) (mg/kg DW), dry matter content (DMC, %) and pH. The sidebar color scale indicates the correlation coefficient r, going from strongest negative correlation (red) to strongest positive correlation (blue). White indicates no correlation at  $p < 0.05$  level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

diversity index (Shannon), and  $^{226}\text{Ra}$  and major anions (SO<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub>) were found in the correlation analysis.

Only *Methanosarcinales* (unclassified) had positive ( $r = 0.803$ ,  $p < 0.01$ ) and *Nitrosotaleaceae* negative ( $r = -0.683$ ,  $p < 0.05$ ) correlation with  $^{226}\text{Ra}$  (Fig. 6A). *Methanocellaceae* (Rice Cluster 1) and *Candidatus Nitrosotalea* correlated positively with sulfate ( $r > 0.68$ ,  $p < 0.04$ ) (Fig. 6A). In addition, several correlations were observed between archaea and phosphate concentrations and pH (Fig. 6A). In addition, the number of archaeal 16S rRNA gene copies correlated negatively ( $r =$

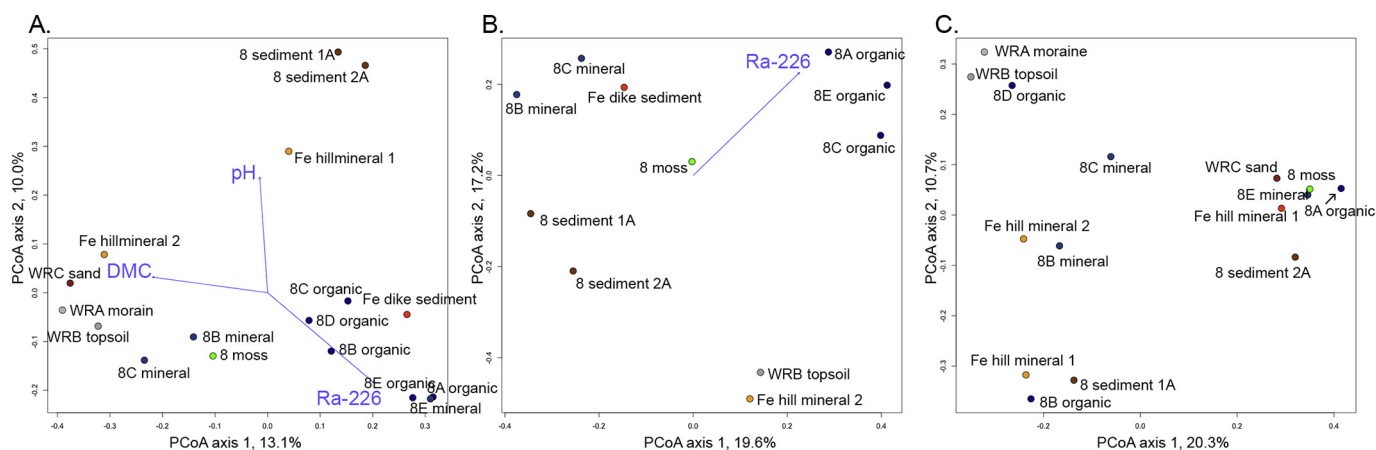
$-0.721$ ,  $p = 0.01$ ) with the dry matter content (%). No correlation between the number of archaeal 16S rRNA gene copies, archaeal richness (Chao1) and diversity (Shannon) and  $^{226}\text{Ra}$  and major anions (SO<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub>) as well as pH were found in the correlation analysis.

The fungal genera *Archaeorhizomyces*, *Oidiodendron*, unclassified *Trichocomaceae*, *Hygrocybe* and *Geminibasidium* correlated negatively and significantly ( $r < -0.55$ ,  $p < 0.04$ ) with  $^{226}\text{Ra}$  (Fig. 6B). The relative abundance of unclassified fungi also correlated negatively and significantly with  $^{226}\text{Ra}$  ( $r = -0.609$ ,  $p = 0.020$ ). In contrast, *Pseudeurotium*



**Fig. 6.** A) Heat map showing Spearman correlation coefficients at  $p < 0.05$  level between major archaeal genera in the Paukkajänvaara area and  $^{226}\text{Ra}$  (Bq g<sup>-1</sup> DW), sulfate and phosphate concentrations (SO<sub>4</sub>, PO<sub>4</sub>) (mg/kg DW), dry matter content (DMC, %) and pH. No significant correlations at the  $p < 0.05$  level for nitrate concentrations were found. B) Heat map showing Spearman correlation coefficients at  $p < 0.05$  level between major fungal genera in the Paukkajänvaara area and  $^{226}\text{Ra}$  (Bq g<sup>-1</sup> DW), nitrate, sulfate and phosphate (NO<sub>3</sub>, SO<sub>4</sub>, PO<sub>4</sub>) concentrations (mg/kg DW), dry matter content (DMC, %) and pH. The sidebar color scale indicates the correlation coefficient r, going from strongest negative correlation (red) to strongest positive correlation (blue). White indicates no correlation at  $p < 0.05$  level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



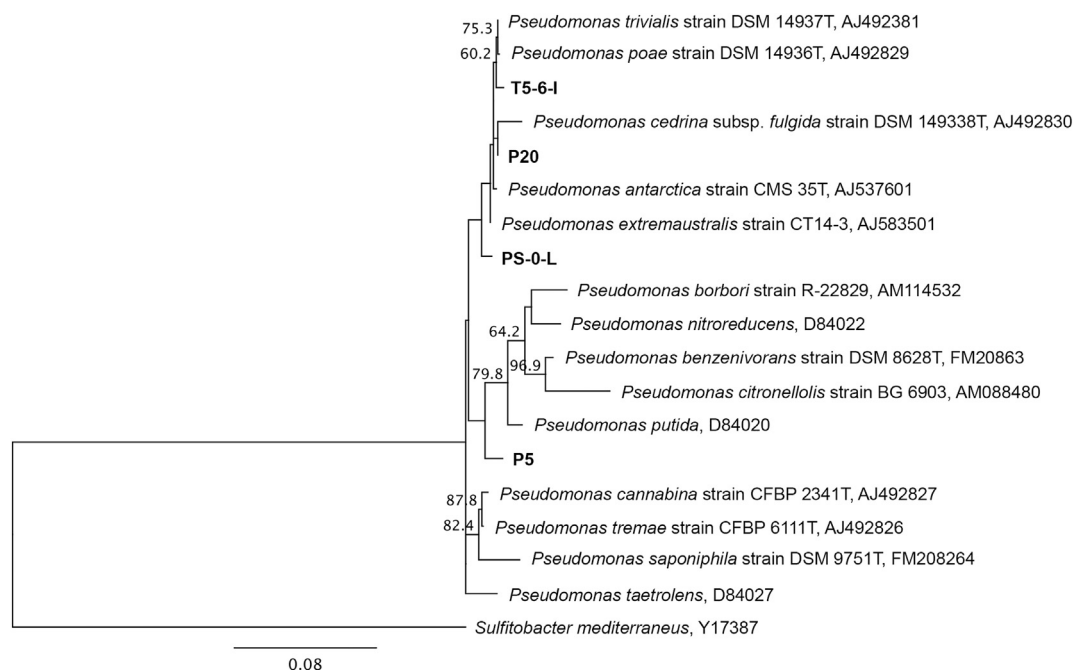


**Fig. 7.** Principal Coordinates Analysis (PCoA) of the A) bacterial, B) archaeal and C) fungal communities in relation to environmental parameters. Vectors for environmental parameters having statistically significant ( $p < 0.05$ ) effect on the microbial communities are shown. In C) no significant environmental parameters were identified.

( $r = 0.600$ ,  $p = 0.023$ ), *Holwaya* ( $r = 0.614$ ,  $p = 0.020$ ), *Tremellomyces* (unclassified) ( $r = 0.574$ ,  $p = 0.032$ ) and *Cryptococcus* ( $r = 0.630$ ,  $p = 0.016$ ) had significant positive correlation with  $^{226}\text{Ra}$ . *Oidiodendron* and *Russula* ( $r < -0.65$ ,  $p < 0.008$ ) also correlated negatively with sulfate concentration. In addition, several correlations between fungal genera and phosphate, nitrate, dry matter content and pH were recorded (Fig. 6B). No correlation between the number of fungal 5.8S rRNA gene copies, fungal richness (Chao1) and diversity (Shannon) major anions ( $\text{SO}_4$ ,  $\text{NO}_3$ ,  $\text{PO}_4$ ) as well as pH and dry matter content (%) were found in the correlation analysis.

The similarity between the microbial community profiles of the different samples was tested using Principal Coordinates analysis (PCoA) and the Bray–Curtis distance model (Fig. 7). The bacterial communities of the mill tailings area organic layer samples (8A, B, C, D, E), one mineral layer sample (8E mineral) and the Fe dike sediment sample clustered together (Fig. 7A).  $^{226}\text{Ra}$  concentrations clearly determined this group. The bacterial communities in the sediment samples from Iso Hiislampi bond (8 sediment 1A and 2A) clustered clearly in a separate

group with Fe hill mineral 1 sample and this group was most strongly determined by pH (Fig. 7A). In addition, the samples from the waste rock pile area (WRA, WRB, WRC), Fe hill mineral 1 and 8B and 8C mineral soil samples clustered together and were clearly strongly affected by dry matter content. In the PCoA analysis of the archaeal communities, the 8A, 8C and 8E organic layer samples from the mill tailings area clustered together (Fig. 7B).  $^{226}\text{Ra}$  concentrations mostly determined this group. In addition, WRB topsoil and Fe hill mineral 2 samples formed a close group and 8 sediment 1A and 8 sediment 2A clustered together. Samples 8C and 8B mineral together with Fe dike sediment also clustered together in the PCoA analysis of the archaeal communities. No significant (at  $p < 0.05$  level) environmental parameters or sample type determined the fungal communities. However, the fungal communities formed three groups. WRA moraine, WRB topsoil and 8D organic samples clearly clustered together and WRC sand, 8 moss, 8E mineral, 8A organic and Fe hill mineral 1 samples formed a close group (Fig. 7C). In addition, 8 sediment 1A, 8B organic and Fe hill mineral 1 clustered together.



**Fig. 8.** Maximum likelihood tree constructed from the 16S rRNA gene sequences of bacterial isolates and relevant reference species. The topology of the tree was tested by bootstrap analyses of 1000 random resamplings and nodes with  $>50\%$  support are indicated. Scale bar represents nucleotide substitutions per site.

**Table 3**  
Biochemical characteristics of *Pseudomonas* spp. strains used in this study.

|  | P5  | P20 | T5-6-I <sup>a</sup> | PS-0-L <sup>a</sup> |
|--|-----|-----|---------------------|---------------------|
| Morphology   | rod | rod | rod                 | rod                 |
| Gram   | -   | -   | -                   | -                   |
| Catalase   |     |     |                     |                     |
| Oxidase  |     |     | -                   | -                   |
| Urease   | -   | -   | -                   | -                   |
| Glucose utilization  |     |     | -                   | -                   |
| Nitrate utilization  |     | -   |                     |                     |
| Arginine   |     | -   | ±                   | ±                   |
| Aliphatic thiol  |     | -   | -                   | -                   |
| Triglyceride   |     |     |                     |                     |
| $\rho$ -Nitrophenyl-phosphoester   |     |     |                     |                     |
| $\rho$ -Nitrophenyl- <i>N</i> -acetyl- $\beta$ , <i>D</i> -glucosaminide | -   | -   | -                   | -                   |
| $\rho$ -Nitrophenyl- $\alpha$ , <i>D</i> -glucoside                      | -   | -   | -                   | -                   |
| $\rho$ -Nitrophenyl- $\beta$ , <i>D</i> -glucoside                       | -   | -   | -                   | -                   |
| Proline- $\beta$ -naphthylamide  |     |     |                     |                     |
| Pyrrolidine- $\beta$ -naphthylamide                                      | -   |     |                     | -                   |
| $\gamma$ -Glutamyl $\beta$ -naphthylamide                                |     |     |                     | -                   |
| Tryptophane $\beta$ -naphthylamide                                       | -   | -   | -                   | -                   |
| <i>N</i> -Benzyl-arginine- $\beta$ -naphthylamide                        |     |     |                     |                     |

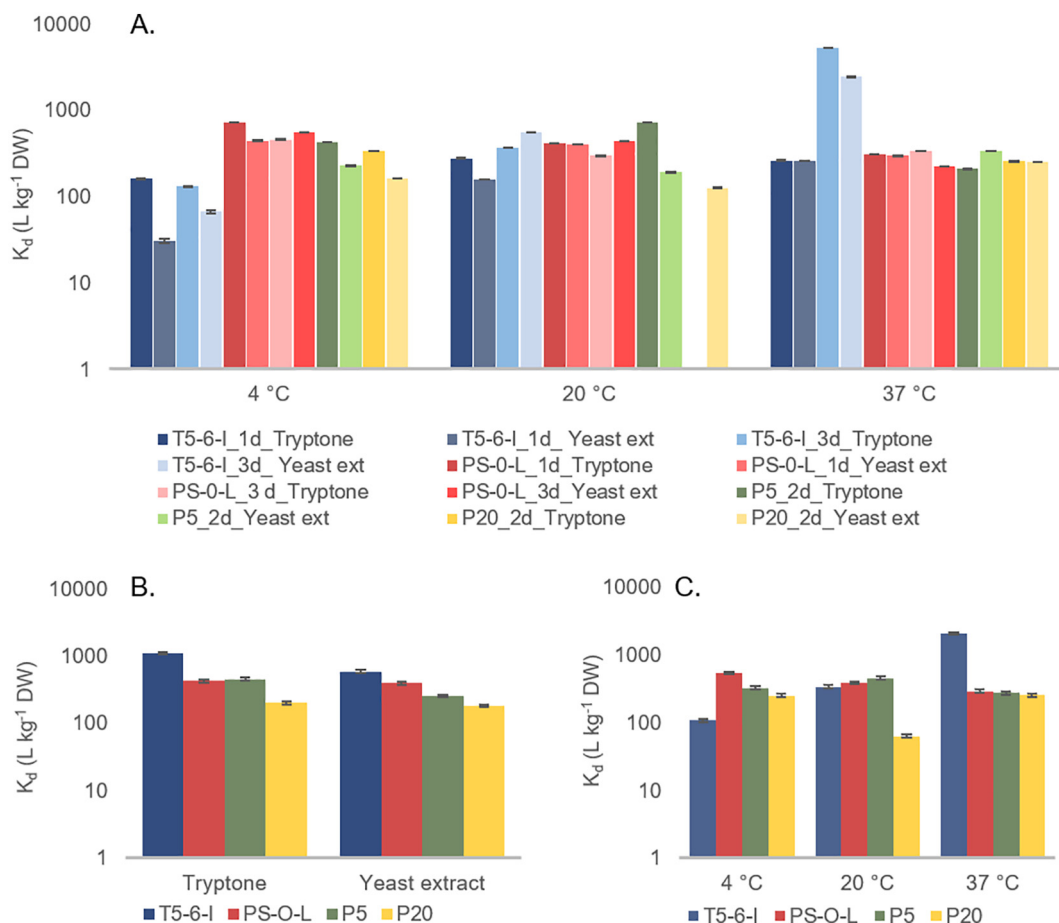
<sup>a</sup> From Lusa et al., 2016.

### 3.6. Characterization of the bacterial isolates and the effects of *Pseudomonas* spp. strains on the Ra uptake and leaching

Altogether 6 different bacterial isolates were obtained from the Paukkajanvaara area samples most closely representing *Serratia*,

*Pseudomonas*, *Bacillus* and *Psychrobacillus*. According to the 16S rRNA gene sequences, two of the bacterial isolates chosen for the further in-vitro uptake experiments, P5 and P20, affiliated with *Pseudomonas* in the phylogenetic analyses, sharing over 99.7% 16S rRNA gene sequence similarity with their closest neighboring counterparts (Fig. 8). Further, the 16S rRNA genes of strains P5 and P20 shared 97.02% sequence homology. The strains had similar substrate utilization profiles according to the RapID™ ONE and RapID™ NF Plus tests (Table 3), although P20 hydrolyzed arginine and aliphatic thiols whereas P5 hydrolyzed Pyrrolidine- $\beta$ -naphthylamide. Previously isolated strains *Pseudomonas* T5-6-I and PS-0-L (Lusa et al., 2016) had quite similar phenotypic characteristics with the new isolates. However, both strains PS-0-L and T5-6-I utilized nitrate, while of the new isolates only P5 did. Isolates P5 and P20 utilized glucose, whereas T5-6-I and PS-0-L did not.

Uptake of  $^{226}\text{Ra}$  was affected by the nutrient broth, incubation time and incubation temperature used (Fig. 9). For all *Pseudomonas* spp. strains, the maximum mean uptake of  $^{226}\text{Ra}$  was found in 1% Tryptone solution. The average uptake of all strains in 1% Tryptone was 1.5 – fold compared to the uptake in the 1% Yeast extract (Fig. 9B). Statistically significant difference between the  $^{226}\text{Ra}$  uptake in the 1% Tryptone solution and in the 1% Yeast extract was however observed only for the *Pseudomonas* sp. P5 strain using one-way ANOVA ( $F_{\text{crit}} = 4.493$ ,  $F = 5.252$ ,  $p = 0.03$ ). For strain T5-6-I, highest radium uptake (5320 L/kg DW) was observed at 37 °C and an increase in the incubation temperature clearly increased  $^{226}\text{Ra}$  uptake in this strain. The average  $K_d$  values at 37 °C were 20-fold, compared to the average  $K_d$  values



**Fig. 9.** A) Ra uptake in 1% Tryptone and 1% Yeast extract solution in *Pseudomonas* spp. T5-6-I, PS-0-L, P5 and P20 at three different temperatures 4 °C, 20 °C and 37 °C. Incubation times of 1 day (1d) and 3 days (3d) for T5-6-I and PS-0-L and 2 days (2d) for P5 and P20 are shown. B) The mean  $K_d$  values of Ra in 1% Tryptone and 1% Yeast extract media combined from all three temperatures and incubation times. C) The mean  $K_d$  values of Ra as a function of temperature combined from all incubation times and both media. All results are the geometric means of three parallel measurements and the error bars indicate the geometric standard deviation of the three measurements.

observed at 4 °C in this strain (average at 4 °C 107 L/kg DW and at 37 °C 2070 L/kg DW). For this strain, the difference in the  $^{226}\text{Ra}$  uptake between the three different temperatures was found statistically significant using one-way ANOVA ( $F_{\text{crit}} = 9.552$ ,  $F = 57.681$ ,  $p = 0.004$ ). In contrast, for the other three strains (PS-0-L, P5 and P20), statistically significant differences in the uptake of  $^{226}\text{Ra}$  between the temperature of 4 °C, 20 °C and 37 °C were not found. However, in strain PS-0-L  $^{226}\text{Ra}$  uptake decreased with increasing temperature from an average of 540 L/kg DW at 4 °C to 290 L/kg DW at 37 °C. For strains P5 and P20, no clear effect of temperature on the average uptake of  $^{226}\text{Ra}$  was observed. In these strains,  $^{226}\text{Ra}$  uptake ranged between 190 and 720 L/kg DW and 0–340 L/kg DW, respectively, depending on incubation temperature and nutrient broth used.

The decrease in pH from  $\text{pH } 7 \pm 0.3$  to  $\text{pH } 6 \pm 0.3$ , decreased the average  $^{226}\text{Ra}$  uptake in *Pseudomonas* sp. P5 from 720 L/kg DW to 160 L/kg DW. Similarly, an increase in pH to  $8 \pm 0.3$  decreased the average  $^{226}\text{Ra}$  uptake to 290 L/kg DW.

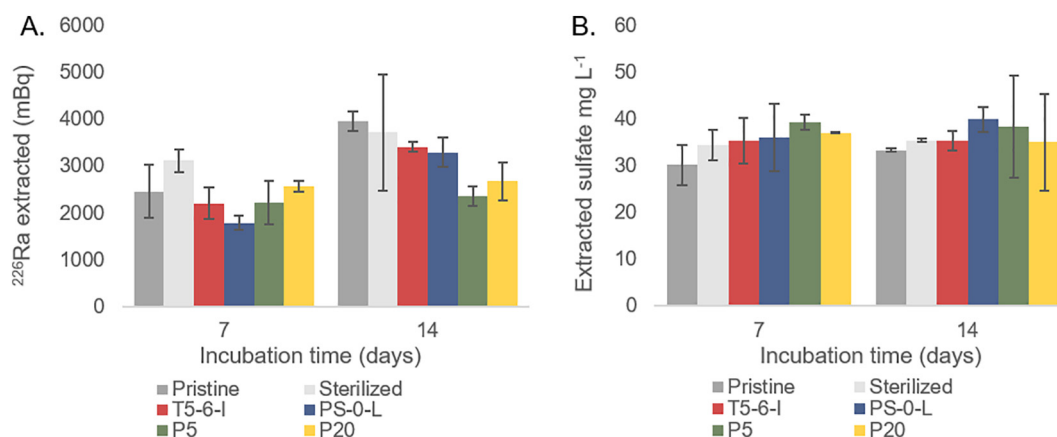
Autoclaved 8E mineral layer sample was incubated with different *Pseudomonas* spp. strains in order to study the effect of these bacteria on the leaching of Ra and sulfate from the Paukkajanvaara soil. Radium leaching from the soil to the solution was observed to increase 1.3-fold in the sterilized samples after 7 days incubation, compared to the sample in which the indigenous microbial population was present (Fig. 10A). After 14 days incubation, only a minor difference between the Ra concentrations extracted from soil was observed between the sample with indigenous microbial community (3950 mBq) and the sterilized sample (3720 mBq). Notable decrease in the Ra extraction between both samples with indigenous microbial community as well as between sterilized sample and the sterilized samples inoculated with *Pseudomonas* spp. strains was however observed on both incubation times (7 days and 14 days) (Fig. 10A). All *Pseudomonas* spp. strains decreased free Ra concentrations in the solution with an average of 23%. However, the decrease was found statistically significant only for the strain P5 when compared to the sterilized samples ( $F_{\text{crit}} = 5.987$ ,  $F = 6.749$ ,  $p = 0.04$ ) using one-way ANOVA.

In contrast to the decreased soluble  $^{226}\text{Ra}$  concentrations after *Pseudomonas* spp. inoculation, an average of 11% increase in the sulfate concentrations in the solution after *Pseudomonas* spp. strains addition, compared to the pristine or sterilized samples was observed (Fig. 10B). At the same time, no significant increase in the solution pH was observed. Similarly to radium, the increase in the solution sulfate concentrations after *Pseudomonas* spp. inoculation was found statistically significant only for the strain P5 using one-way ANOVA ( $F_{\text{crit}} = 18.512$ ,  $F = 30.929$ ,  $p = 0.03$ ).

#### 4. Discussion

Microbiological processes can alter the solubility and bioavailability of radionuclides and thus influence the overall behavior of radionuclides in the environment. At the same time, radionuclides and low pH, typical for the mining areas, can shift the soil microbial communities towards more resistant lineages able to survive in these extremely harsh environments. In the present study, several factors, including variable  $^{226}\text{Ra}$  concentrations, sulfate and nitrate concentrations, pH, sample type and sampling area were found to affect the microbial community profiles in the Paukkajanvaara mine and high bacterial, archaeal and fungal diversity was detected in the area.

The observed  $^{226}\text{Ra}$  activities varied considerably and highest activities were found in the area in which the mill tailings originating from the ore processing were placed during the mining operations. The area is limited to the Iso Hiislampi pond in the North and to the small Fe dike stream flowing from the waste rock pile area, in the East. According to previous studies on the Paukkajanvaara site, vertical leaching of  $^{226}\text{Ra}$  from the tailings repository is likely and was suggested as a possible  $^{226}\text{Ra}$  contamination pathway in this area (Tuovinen et al., 2016). This same phenomenon would well explain also the  $^{226}\text{Ra}$  activities observed in our study. Radium is typically poorly soluble in the environment, but increasing soil acidity may contribute to its improved mobility (Frissel and Köster, 1990). The pH values of the mill tailings area samples varied between 3.6 and 4.2, and pH values below 4.0 were found in the majority of the mill tailings area samples. Higher pH values >4.0 were however observed in the samples with high  $^{226}\text{Ra}$  concentrations and the increase in pH could therefore well explain the increased radium concentrations in these samples. This is due to the somewhat higher pH, compared to the surroundings, that could have immobilized  $^{226}\text{Ra}$  specifically in these points. In addition to low pH, the highest sulfate concentrations were observed in the mill tailings area samples. As radium can precipitate as  $\text{RaSO}_4$  or coprecipitate with barium as  $(\text{Ba,Ra})\text{SO}_4$  in areas with moderate or high sulfate concentrations, in addition to pH, sulfate may acts as the major solubility-controlling factor of radium in this area contributing to the formation of radium hot-spots. Radium may however be re-solubilized by sulphate reducing bacteria (SRB), such as *Desulfovibrio vulgaris*, in the presence of useable carbon source. SRB were found in the Paukkajanvaara mine area, even though in low relative abundances (<1% of bacterial sequence reads in all samples). The highest relative abundance (in total 0.4% of the bacterial sequence reads) of SRB (mostly represented by *Desulfobacterales*) was observed in the Iso Hiislampi pond sediment with relatively low  $^{226}\text{Ra}$  concentrations of  $1.6 \text{ Bq g}^{-1}\text{DW}$ . In addition, *Desulfosporosinus* were detected at low relative abundances (<1% of all



**Fig. 10.** A) Ra and B) sulfate extracted from the 8E mineral soil after 7 and 14 days of incubation with indigenous microorganisms, in sterilized sample and in samples inoculated with *Pseudomonas* spp. strains T5-6-L, PS-0-L, P5 and P20. All results are the means of three parallel determinations and the error indicated is the standard deviation of the three determinations.



bacterial reads) in the Paukkajanvaara area samples. Further, *Methanocellaceae* Rice\_Cluster\_I showed a strong positive correlation with sulfate concentrations. This may reflect the syntrophic relationship between certain methanogenic archaea and SRB in the Paukkajanvaara mine area. In addition to the autotrophic bacteria, the dissolution of Ra by heterotrophic microorganisms is also possible due to the production of organic acid metabolites, which in turn decrease the pH.

On the other hand many bacteria, including many species of *Xanthobacteraceae* and *Pseudomonadaceae*, are capable of oxidizing reduced sulfur species (increasing sulfate concentrations), and e.g. facultative chemolithoautotrophy with hydrogen and/or reduced sulfur compounds as electron donor and energy source is found in many species of *Xanthobacteraceae* (Oren, 2014). *Xanthobacteraceae* were present at high relative abundances in all Paukkajanvaara mine area samples. Statistically significant correlations between the sulfate concentrations and bacterial genera belonging to *Xanthobacteraceae* were, however, not found in our study. Contrary to expected, the highest relative abundance of *Xanthobacteraceae* was observed in the samples from the waste rock pile area, with sulfate concentrations below the limit of detection. In contrast, in the Iso Hiislampi pond sediment, with increased concentration of sulfate, only a low relative abundance (< 1% of all bacteria reads) of *Xanthobacteraceae* were detected. At the same time, their relative abundance was quite high, in total 6.8% of the bacterial community, in the Fe dike sediment with sulfate concentrations below the limit of detection. It should be noted, however, that *Xanthobacteraceae* were also found in considerable amounts, from 2.4% to 10% of all bacterial reads, in the mill tailings area samples with both high and low sulfate concentrations, and therefore the relationship between the *Xanthobacteraceae* and the sulfate content in different samples and sampling areas/points cannot be fully resolved. Surprisingly, *Mycobacterium* correlated negatively with the sulfate concentrations. This was not expected, as *Mycobacteria* (even though mainly the human pathogens like *M. tuberculosis*) are known to synthesize sulfated metabolites to survive hostile environments (Schelle and Bertozzi, 2006).

The chemical analyses of the Paukkajanvaara area soil samples showed additional substantial variation in the concentration of nitrate and phosphate in the area. Clearly, the highest nitrate concentration was observed in the moss sample taken from the mill tailings area. Mosses play an important role in N-cycling by addition of N to the ecosystem via N-fixing associations with cyanobacteria (DeLuca et al., 2002). In our study, the proportion of cyanobacteria was over all low, but several correlations between other bacterial genera and nitrate were nevertheless found. Previous genomic analyses suggest that *Acidobacteria* have important roles in the N-cycling in soils and sediments and that these bacteria would be able to reduce nitrate and nitrite (Ward et al., 2009). *Acidobacteria* were found in high relative abundances in all Paukkajanvaara area samples. However, of the *Acidobacterial* phylum, only negative correlation between genera *Occallatibacter* and nitrate was observed. In contrast, *Rhizobiales*, comprising of several nitrogen-fixing genera, had strong positive correlation with nitrate. In addition, archaea involved in the nitrogen cycle, especially ammonium oxidizing *Nitrosotaleaceae* (Candidatus *Nitrosotalea*), were present in high relative abundance in the sample with very high nitrate concentration.

Throughout the tested Paukkajanvaara mine area, the bacterial community was dominated by  $\alpha$ - and  $\gamma$ -*Proteobacteria* and the communities appeared more similar in the samples from the more similar locations (i.e. tailing, waste rock and Iso Hiislampi bond areas). *Pseudomonaceae* (mostly represented by *Pseudomonas*), *Xanthobacteraceae* (mainly represented by *Pseudolabrys* and unclassified/uncultured *Xanthobacteraceae*) and *Burkholderiaceae* (mainly represented by *Burkholderia*-*Caballeronia*-*Paraburkholderia*) dominated the *Proteobacterial* community in the mine area. In fact, *Pseudomonas* and *Burkholderia* were the most common individual

bacterial genera in the area. *Pseudomonaceae* tolerate a variety of physical conditions, exhibit wide metabolic diversity and subsequently are able to colonize a wide range of ecological niches (Madigan and Martinko, 2005). In present study, all tested *Pseudomonas* spp. strains removed Ra from the solution and the uptake was affected by the nutrient broth, incubation time and incubation temperature. Interestingly, as sterilized soil samples were inoculated with *Pseudomonas* spp. strains P5, P20, T5-6-I and PS-0-L, on average 23% more Ra was retained in the soil mineral phase, compared to the sample, without bacterial inoculation. The decrease in the mobilized Ra fraction after bacterial inoculation was apparently caused by the uptake of Ra from the solution by the bacterial biofilms formed by *Pseudomonas* spp. strains on the mineral surfaces of the soil samples. Simultaneously to the decrease in mobilized Ra, the addition of *Pseudomonas* spp. strains increased sulfate concentrations in the leaching solution, presumably through microbiological oxidation of reduced sulfur species found in the soil. Microbiological oxidation of reduced sulfur species to sulfate is one of the most important reactions of the environmental sulfur cycle (Friedrich et al., 2001) and may well be performed by *Pseudomonas*, which also include known sulfur oxidizing species (Friedrich and Mitrenga, 1981).

Further research will show whether other *Pseudomonas* spp. share the features discovered here. Data of this type will be of prime importance for understanding the effects of microbial processes in soil environment related to the radionuclide environmental behavior.

## 5. Conclusions

Paukkajanvaara mine area had high bacterial, archaeal and fungal diversity and several bacterial groups appeared adapted to the elevated radiation in their surroundings, giving them a competitive edge in this environment. *Methanosarcinales* and several fungal genera correlated positively with <sup>226</sup>Ra, whereas other microbial groups appeared susceptible to elevated radiation.

Sulfate and pH concentrations appeared to control the solubility of radium and may cause precipitated radium hot-spots. *Pseudomonas* spp. strains took up radium and simultaneously increased sulfate concentrations in the leached solution. *Pseudomonas* were common in the majority of the Paukkajanvaara area samples and may thus affect the immobilization-mobilization behavior of Ra in this area.

## Declaration of Competing Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

## Acknowledgments

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## CRediT author statement

Merja Lusa: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Writing – Original Draft, Writing – Reviewing and Editing, Supervision, Project Administration. Jenna Knuutinen: Investigation. Marcus Lindgren: Investigation. Juhani Virkanen: Investigation. Malin Bomberg: Conceptualization, Methodology, Investigation, Writing- Reviewing and Editing.

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## Appendix 1

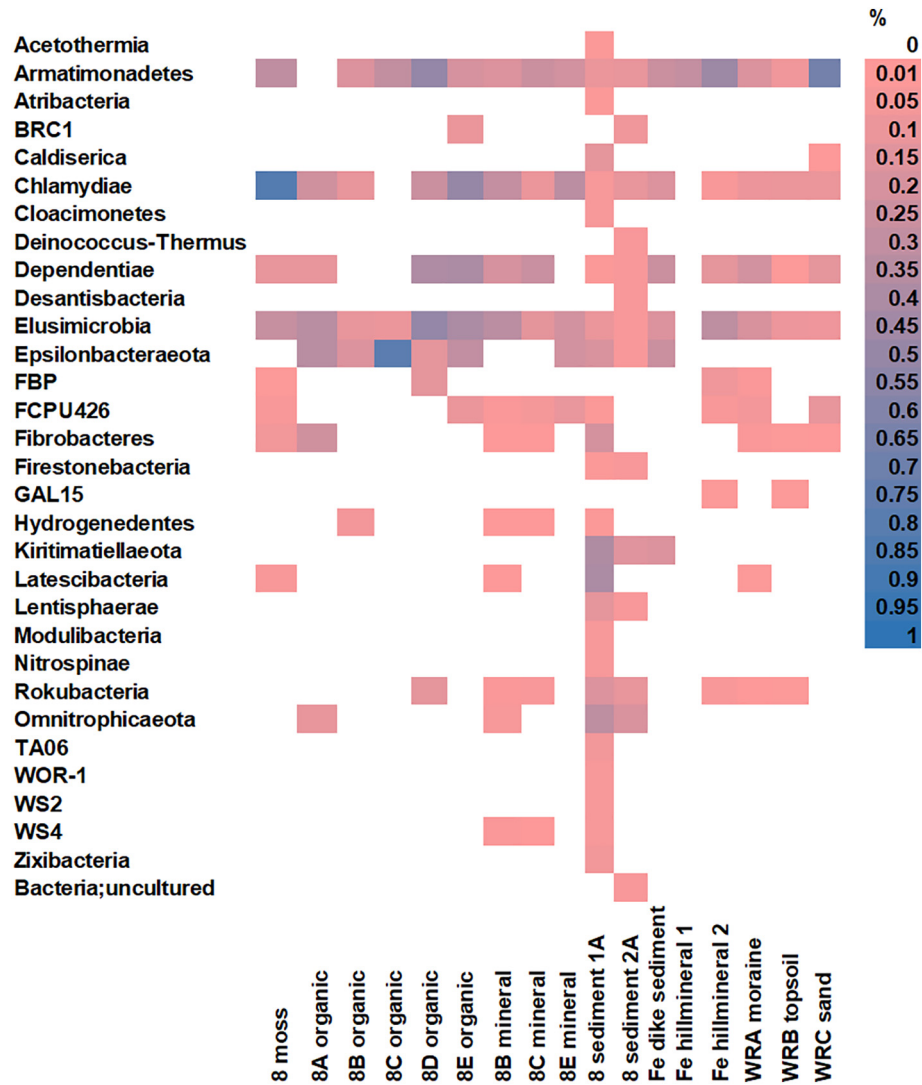
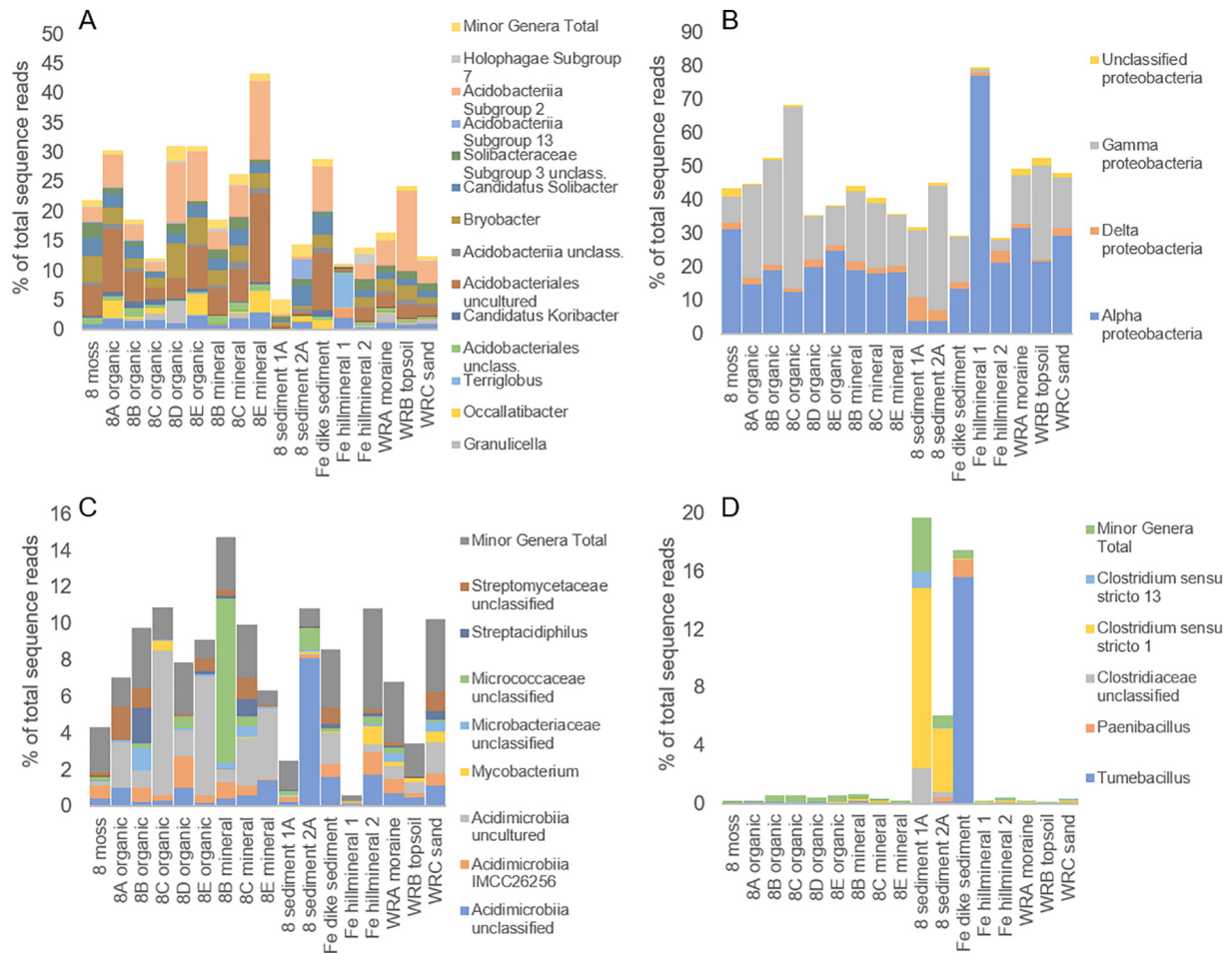


Fig. A1. Heatmap showing the relative abundance of the bacterial phyla representing at <1% of the bacterial sequence reads in Paukkajanvaara samples.

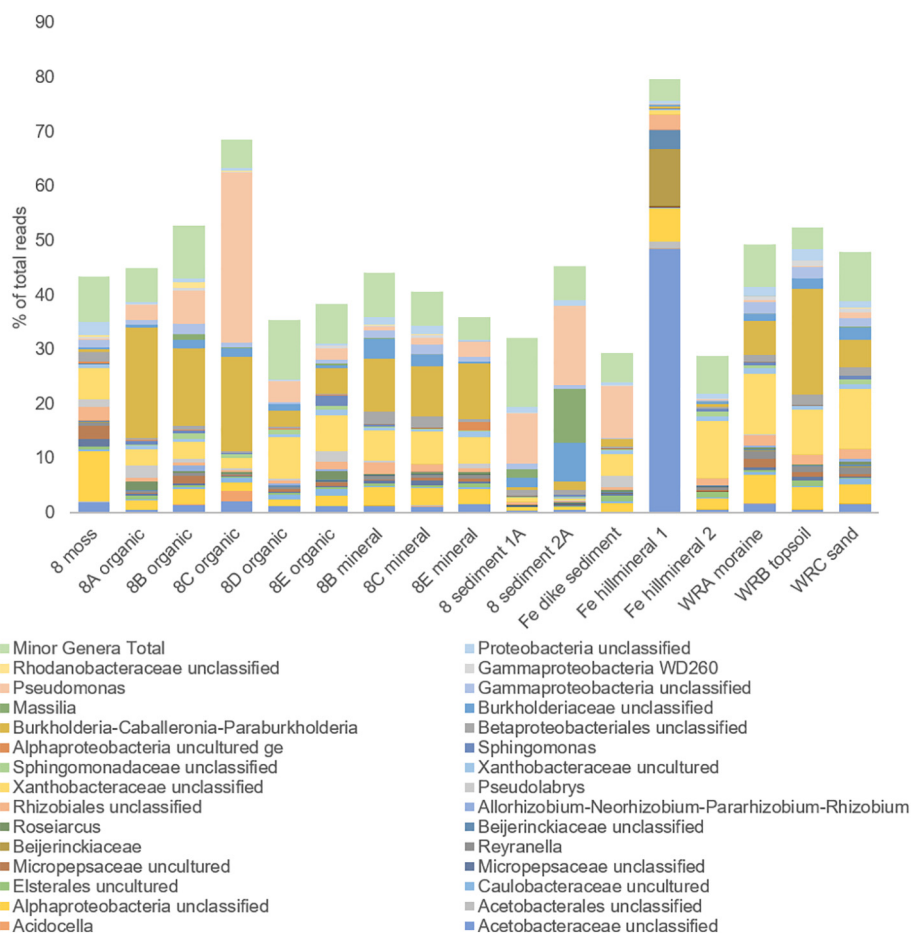


Fig. A2. Heatmap showing the relative abundance of the major bacterial genera of relative abundances >1% at least in one of the samples (% of sequence reads) in Paukkajanvaara area.





**Fig. A3.** The relative abundance of classes representing the most prevailing bacterial phyla in Paukkajanvaara mine area samples, A) Acidobacteria, B) Proteobacteria, C) Actinobacteria, D) Firmicutes.



**Fig. A4.** The relative abundance of Proteobacterial genera in Paukkajanvaara mine area samples. The group 'Minor genera' contain the combined relative abundance of genera contributing with <1% of the bacterial community in any sample.

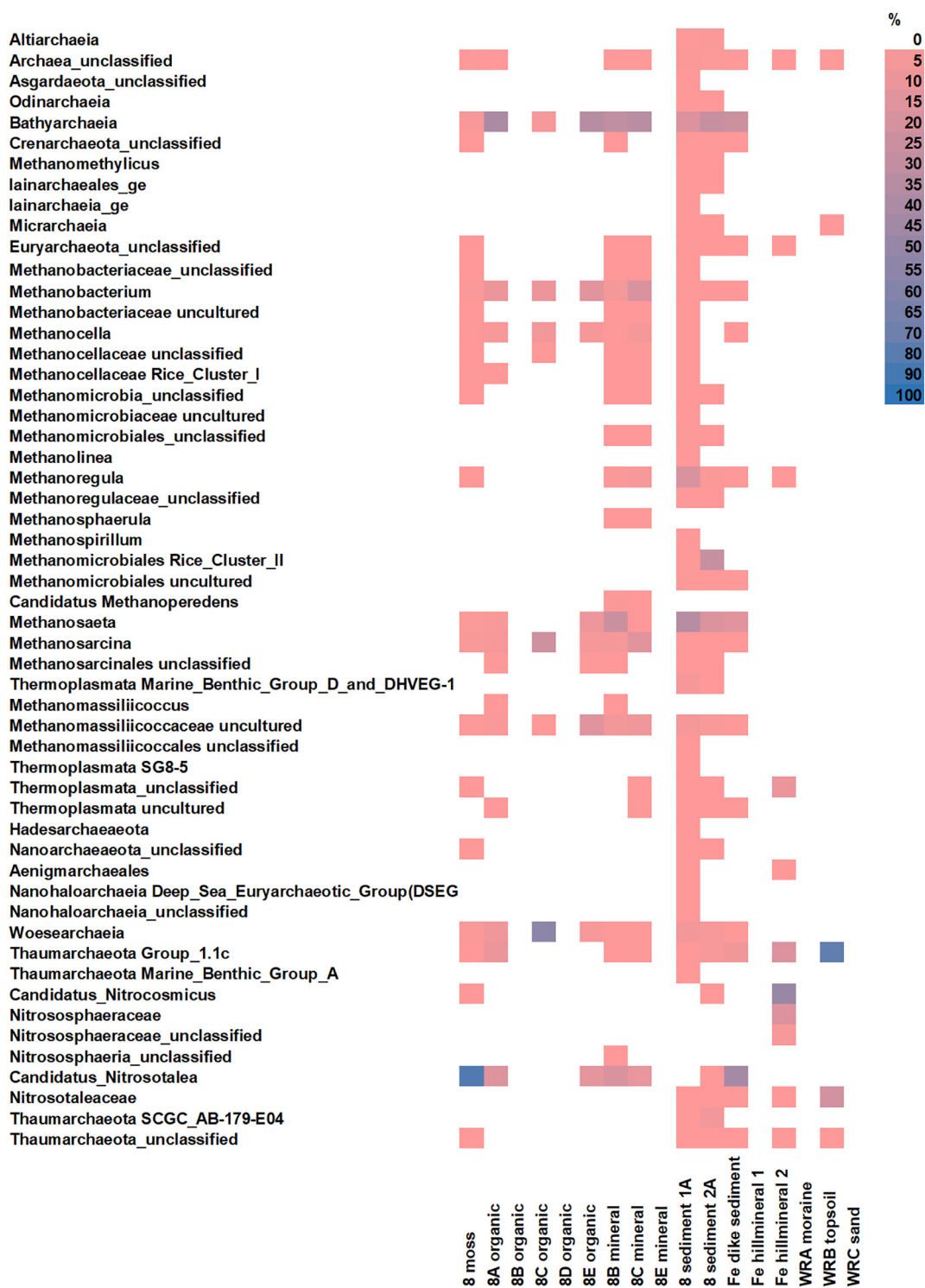


Fig. A5. Heatmap showing the relative abundance of the archaeal genera (% of sequence reads) in Paukkajanvaara area.



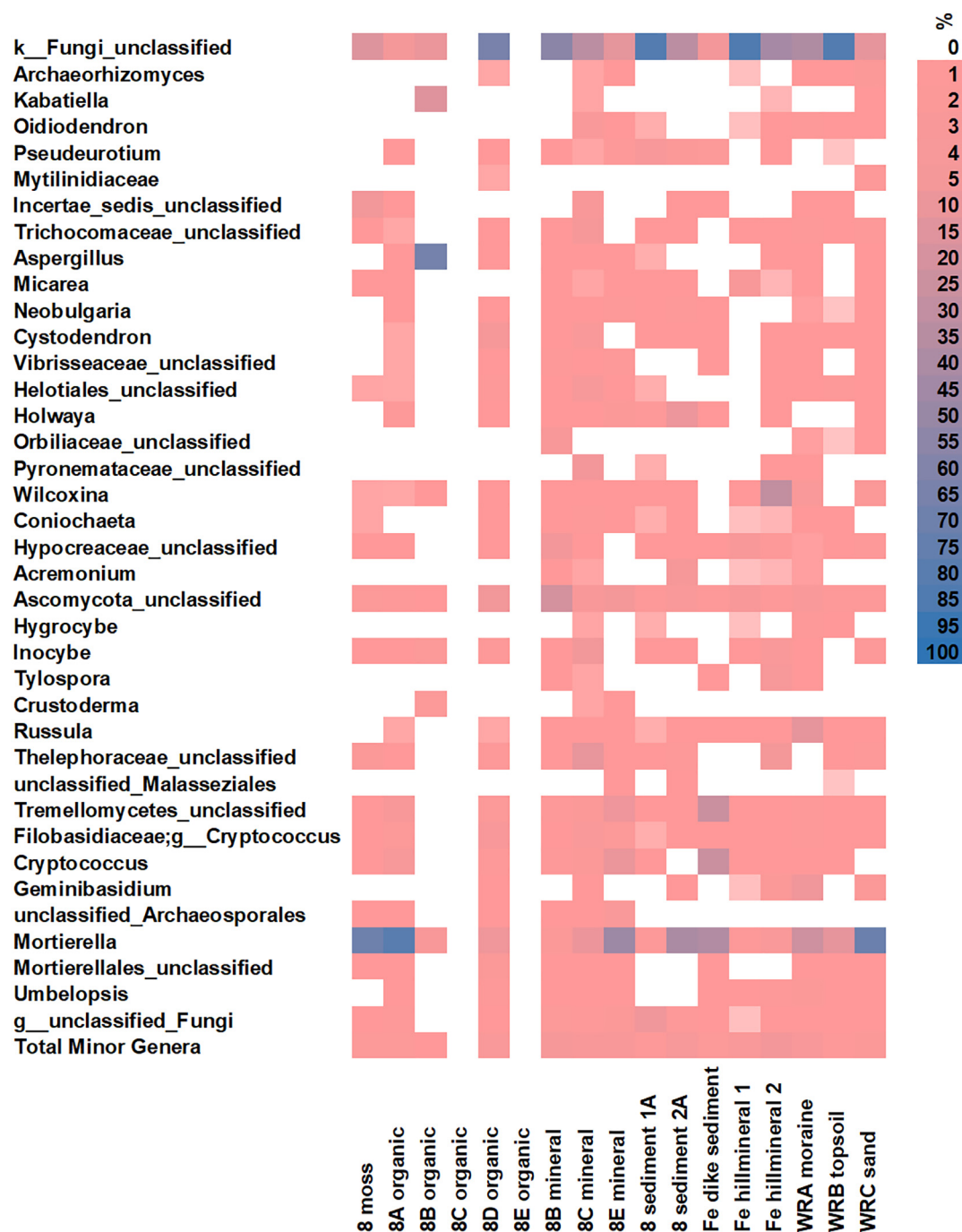


Fig. A6. Heatmap showing the relative abundance of the fungal genera (% of sequence reads) in Paukkajanvaara area.

Table A1

Observed total number of bacterial 16S rRNA gene sequence reads (Sequences), observed (OTUs) and estimated number of bacterial OTUs (Chao1) of OTUs, OTU coverage (%) estimated from the Chao1 (Coverage) and diversity indices (Shannon). The data was rarefied to 2000 sequences for calculating the number of OTUs, Shannon index, Chao1 estimate and Good's coverage, when the original number of sequences was >2000. The coverage describes the percentage of OTUs detected from the rarefied data set compared to the Chao1 estimated number of OTUs determined from the rarefied data.

| Sample     | Sequences | OTUs | Diversity | Richness | Coverage (%) |
|------------|-----------|------|-----------|----------|--------------|
|            |           |      | Shannon   | Chao1    |              |
| 8 moss     | 6207      | 1710 | 7.3       | 13,049   | 23           |
| 8A organic | 881       | 549  | 5.8       | 2353     | 49           |
| 8B organic | 1748      | 1043 | 6.5       | 4126     | 53           |
| 8C organic | 1016      | 490  | 5.4       | 1762     | 63           |
| 8D organic | 773       | 569  | 6.2       | 2338     | 40           |
| 8E organic | 998       | 649  | 6.2       | 2503     | 49           |
| 8B mineral | 6998      | 1363 | 6.8       | 8414     | 41           |
| 8C mineral | 8263      | 1512 | 7.1       | 10,253   | 34           |

**Table A1** (continued)

| Sample            | Sequences | OTUs | Diversity | Richness | Coverage (%) |
|-------------------|-----------|------|-----------|----------|--------------|
|                   |           |      | Shannon   | Chao1    |              |
| 8E mineral        | 916       | 583  | 6.0       | 2721     | 47           |
| 8 sediment 1A     | 4735      | 1314 | 6.5       | 8567     | 42           |
| 8 sediment 2A     | 2674      | 1011 | 6.2       | 5063     | 59           |
| Fe dike sediment  | 1167      | 657  | 5.9       | 2190     | 56           |
| Fe hill mineral 1 | 3412      | 708  | 5.6       | 2609     | 74           |
| Fe hill mineral 2 | 6352      | 1382 | 6.9       | 7421     | 42           |
| WRA moraine       | 10,692    | 1586 | 7.2       | 10,375   | 30           |
| WRB topsoil       | 6694      | 1378 | 6.9       | 8449     | 41           |
| WRC sand          | 6941      | 1398 | 6.9       | 7633     | 41           |

**Table A2**

Observed number of archaeal 16S rRNA gene sequence reads (Sequences), observed (OTUs) and estimated number of archaeal OTUs (Chao1) of OTUs, OTU coverage (%) estimated from the Chao1 (Coverage) and diversity indices (Shannon). The data was rarefied to 1000 sequences for calculating the number of OTUs, Shannon index, Chao1 estimate and Good's coverage, when the original number of sequences was >1000. The coverage describes the percentage of OTUs detected from the rarefied data set compared to the Chao1 estimated number of OTUs determined from the rarefied data.

| Sample            | Sequences | OTUs | Diversity | Richness | Coverage (%) |
|-------------------|-----------|------|-----------|----------|--------------|
|                   |           |      | Shannon   | Chao1    |              |
| 8 moss            | 2923      | 106  | 2.1       | 235      | 94           |
| 8A organic        | 368       | 89   | 3.8       | 172      | 87           |
| 8B organic        | 32        |      |           |          |              |
| 8C organic        | 164       | 39   | 3.1       | 58       | 89           |
| 8D organic        | 91        |      |           |          |              |
| 8E organic        | 126       | 49   | 3.5       | 96       | 78           |
| 8B mineral        | 4061      | 172  | 4.0       | 374      | 91           |
| 8C mineral        | 2110      | 143  | 3.6       | 317      | 92           |
| 8E mineral        | 12        |      |           |          |              |
| 8 sediment 1A     | 6913      | 360  | 5.0       | 1067     | 75           |
| 8 sediment 2A     | 7468      | 269  | 4.5       | 716      | 83           |
| Fe dike sediment  | 1112      | 165  | 3.8       | 311      | 92           |
| Fe hill mineral 1 | 1         |      |           |          |              |
| Fe hill mineral 2 | 1913      | 72   | 2.4       | 127      | 97           |
| WRA moraine       | 66        |      |           |          |              |
| WRB topsoil       | 277       | 35   | 2.5       | 52       | 94           |
| WRC sand          | 12        |      |           |          |              |

**Table A3**

Observed number of fungal ITS1 region sequence reads (Sequences), observed (OTUs) and estimated number of fungal OTUs (Chao1) of OTUs, OTU coverage (%) estimated from the Chao1 (Coverage) and diversity indices (Shannon). The data was rarefied to 1000 sequences for calculating the number of OTUs, Shannon index, Chao1 estimate and Good's coverage, when the original number of sequences was >1000. The coverage describes the percentage of OTUs detected from the rarefied data set compared to the Chao1 estimated number of OTUs determined from the rarefied data.

| Sample            | Sequences | OTUs | Diversity | Richness | Coverage (%) |
|-------------------|-----------|------|-----------|----------|--------------|
|                   |           |      | Shannon   | Chao1    |              |
| 8 moss            | 3292      | 157  | 3.5       | 355      | 91           |
| 8A organic        | 3345      | 208  | 4.1       | 457      | 88           |
| 8B organic        | 263       | 69   | 3.4       | 115      | 87           |
| 8C organic        | 27        |      |           |          |              |
| 8D organic        | 3307      | 301  | 4.6       | 783      | 80           |
| 8E organic        | 0         |      |           |          |              |
| 8B mineral        | 2911      | 288  | 4.7       | 740      | 82           |
| 8C mineral        | 3299      | 306  | 4.8       | 776      | 80           |
| 8E mineral        | 1804      | 269  | 4.6       | 649      | 84           |
| 8 sediment 1A     | 3597      | 322  | 4.8       | 777      | 79           |
| 8 sediment 2A     | 2446      | 255  | 4.5       | 551      | 85           |
| Fe dike sediment  | 1865      | 197  | 4.0       | 391      | 89           |
| Fe hill mineral 1 | 4573      | 211  | 4.0       | 608      | 87           |
| Fe hill mineral 2 | 3989      | 236  | 4.0       | 731      | 83           |
| WRA moraine       | 3079      | 283  | 4.5       | 787      | 81           |
| WRB topsoil       | 4868      | 105  | 2.2       | 280      | 94           |
| WRC sand          | 2748      | 209  | 3.7       | 545      | 86           |

## References

- Äikäs, O., 2012. Koli U. Geol. Surv. Finland 53, 254–255 Special Paper.
- Bhattacharyya, D., 1998. Issues in the disposal of waste containing naturally occurring radioactive material. *Appl. Radiat. Isot.* 49, 215–226.
- Bomberg, M., Lamminmäki, T., Itävaara, M., 2016. Microbial communities and their predicted metabolic characteristics in deep fracture groundwaters of the crystalline bedrock at Olkiluoto, Finland. *Biogeosciences* 13, 6031–6047.
- Carvalho, F.P., Oliveira, J.M., Malta, M., 2014. Intake of radionuclides with the diet in uranium mining areas. *Procedia Earth and Planetary Science* 8, 43–47.
- Chen, L.X., Huang, L.-N., Méndez-García, C., Kuang, J.-L., Hua, Z.-S., Liu, J., et al., 2016. Microbial communities, processes and functions in acid mine drainage ecosystems. *Curr. Opin. Biotechnol.* 38, 150–158.
- Darko, E., Faanu, A., Awudu, A., Emi-Reynolds, G., Yeboah, J., Oppon, O., Akaho, E., 2009. Public exposure to hazards associated with natural radioactivity in open-pit mining in Ghana. *Radiat. Prot. Dosim.* 138, 45–51.

- DeLuca, T.H., Zackrisson, O., Nilsson, M.-C., Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature* 419, 917–920.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32 (5), 1792–1797.
- Friedrich, C.G., Mitrenga, G., 1981. Oxidation of thiosulfate by *Paracoccus denitrificans* and other hydrogen bacteria. *FEMS Microbiol. Lett.* 10, 209–212.
- Friedrich, C.G., Rother, D., Bardischewsky, F., Quentmeier, A., Fischer, J., 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? *Appl. Environ. Microbiol.* 67 (7), 2873–2882.
- Frissel, M., Köster, H., 1990. Radium in soil. The environmental behaviour of radium. Technical Reports Series No. 310. IAEA, Vienna, pp. 323–334.
- Galitskaya, P., Biktasheva, L., Saveliev, A., Ratering, S., Schnell, S., Selivanovskaya, S., 2015. Response of soil microorganisms to radioactive oil waste: results from a leaching experiment. *Biogeosciences* 12, 3681–3693.
- Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118.
- Haugland, R., Vesper, S., (2002) US Environmental Protection Agency, 2002. Method of identifying and quantifying specific fungi and bacteria. U.S. Patent 6,387,652.
- Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000km salinity gradient of the Baltic Sea. *ISME J.* 5 (10), 1571–9. <https://doi.org/10.1038/ismej.2011.41> (Epub 2011 Apr 7).
- Herranz, M., Idoeta, R., Abelaïras, A., Legarda, F., 2006. Determination of Ra-224, Ra-226 and Ra-228 by Gamma-ray Spectrometry With Radon Retention. International Atomic Energy Agency (IAEA).
- Huang, L.-N., Kuang, J.-L., Shu, W.-S., 2016. Microbial ecology and evolution in the acid mine drainage model system. *Trends Microbiol.* 24, 581–593.
- Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. *Mammalian Protein Metabolism* 3 (21), 132.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* 41, e1.
- Köljal, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bate, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Pöldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiß, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of Fungi. *Mol. Ecol.* <https://doi.org/10.1111/mec.12481>.
- Kuang, J., Huang, L., He, Z., Chen, L., Hua, Z., Jia, P., 2016. Predicting taxonomic and functional structure of microbial communities in acid mine drainage. *ISME J.* 10, 1527–1539.
- Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), *Nucleic Acid Techniques in Bacterial Systematics*. John Wiley & Sons, Inc., New York, U.S.A., pp. 115–176.
- Li, F., Liu, M., Li, Z., Jiang, C., Han, F., Che, Y., 2013. Changes in soil microbial biomass and functional diversity with a nitrogen gradient in soil columns. *Appl. Soil Ecol.* 64, 1–6.
- Liang, G., Yang, F., Yu, D., 2010. MicroRNA395 mediates regulation of sulfate accumulation and allocation in *Arabidopsis thaliana*. *Plant J.* 62 (6), 1046–1057.
- Lusa, M., Bomberg, M., Aromaa, H., Knuutinen, J., Lehto, J., 2015. Sorption of radioiodide in an acidic, nutrient-poor boreal bog: insights into the microbial impact. *J. Environ. Radioact.* 143, 110–122.
- Lusa, M., Lehto, J., Aromaa, H., Knuutinen, J., Bomberg, M., 2016. The uptake of radioiodide by *Paenibacillus* sp., *Pseudomonas* sp., *Burkholderia* sp. and *Rhodococcus* sp. isolated from a boreal nutrient-poor bog. *J. Environ. Sci.* 44, 26–37.
- Lusa, M., Knuutinen, J., Bomberg, M., 2017. Uptake and reduction of Se(IV) in two heterotrophic aerobic *Pseudomonas* strains isolated from boreal bog environment. *AIMS Microbiology* (4), 798–814.
- Madigan, M., Martinko, J. (Eds.), 2005. *Brock Biology of Microorganisms*, 11th ed. Prentice Hall. ISBN: 0-13-144329-1 ISBN.
- Marcin, C., Marcin, G., Justyna, M.P., Katarzyna, K., Maria, N., 2013. Diversity of microorganisms from forest soils differently polluted with heavy metals. *Appl. Soil Ecol.* 64, 7–14.
- Marin, J.A., Hernandez, T., Garcia, C., 2005. Bioremediation of oil refinery sludge by landfarming in semiarid conditions: influence on soil microbial activity. *Environ. Res.* 98, 185–195.
- Mason, C.F., 2002. *Biology of Freshwater Pollution*. Longman Scientific and Technical, Essex.
- McMurdie, Holmes, 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8 (4), e61217.
- Méndez-García, C., Peláez, A.I., Mesa, V., Sánchez, J., Golyshina, O.V., Ferrer, M., 2015. Microbial diversity and metabolic networks in acid mine drainage habitats. *Front. Microbiol.* 6, 475. <https://doi.org/10.3389/fmicb.2015.00475>.
- Mondani, L., Benzerara, K., Carrière, M., Christen, R., Mamindy-Pajany, Y., Février, L., et al., 2011. Influence of uranium on bacterial communities: a comparison of natural uranium-rich soils with controls. *PLoS One* 6 (10), e25771. <https://doi.org/10.1371/journal.pone.0025771>.
- Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Köljal, U., Abarenkov, K., 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* <https://doi.org/10.1093/nar/gky1022>.
- Oren, A., 2014. The family *Xanthobacteraceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), *The Prokaryotes*. Springer, Berlin, Heidelberg.
- Paschoa, A., 1998. Potential environmental and regulatory implications of naturally occurring radioactive material (NORM). *Appl. Radiat. Isot.* 49, 189–196.
- Piirainen, T., 1968. Die Petrologie und die Uranlagerstätten de Koli-Kaltimogebiets im finnischen Nordkarelien. 237. Bulletin de la commission géologique de Finlande, p. 99.
- Pohjolainen, E., 2015. Uranium deposits in Finland. In: Maier, W.D., O'Brien, H., Lahtinen, R. (Eds.), *Mineral Deposits of Finland*. Elsevier, Amsterdam, pp. 659–683.
- Prenafeta Boldú, F., Summerville, R., de Boer, W., Boschker, H., Gams, W., 2014. Biodiversity and ecology of soil fungi in a primary succession of a temperate coastal dune system. *Nova Hedwigia* 99, 347–372. <https://doi.org/10.1127/0029-5035/2014/0203>.
- Pruesse, E., Quast, C., Knittel, K., Fuch, S.B., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nuc. Acids Res.* 35, 7188–7196.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41 (D1), D590–D596. <https://doi.org/10.1093/nar/gks1219> Nov 27.
- R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL: <http://www.R-project.org/>.
- Rajala, P., Bomberg, M., Kietäväinen, R., Kukkonen, I., Ahonen, L., Nyyssönen, M., Itävaara, M., 2015. Deep subsurface microbes rapidly reactivate in the presence of C-1 compounds. *Microorganisms* 3, 17–33.
- Robb, G.A., 1994. Environmental consequences of coal-mine closure. *Geogr. J.* 160, 33–40.
- Satvatmanesh, D., Siavoshi, F., Beitollahi, M.M., Amidi, J., Fallahian, N., 2003. Biosorption of <sup>226</sup>Ra in high level natural radiation areas of Ramsar, Iran. *J. Radioanal. Nucl. Chem.* 258 (3), 483–486.
- Saunders, J.A., Pivetz, B.E., Voorhies, N., Wilkin, R.T., 2016. Potential aquifer vulnerability in regions down-gradient from uranium in situ recovery (ISR) sites. *J. Environ. Management* 183 (1), 67–83.
- Schelle, M.W., Bertozzi, C.R., 2006. Sulfate metabolism in mycobacteria. *ChemBiochem* 7 (10), 1516–1524.
- Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541.
- Strandberg, G.W., Shumate, S.E., Parrott Jr., J.R., North, S.E., 1981. Microbial Accumulation of Uranium, Radium, and Cesium. 618. NBS Spec. Publ. U.S., pp. 274–285.
- Tejada, M., Gonzalez, J.L., Hernandez, M.T., Garcia, C., 2008. Application of different organic amendments in a gasoline contaminated soil: effect on soil microbial properties. *Bioresour. Technol.* 99, 2872–2880.
- Tsezos, M., Keller, D.M., 1983. Adsorption of radium-226 by biological origin absorbents. *Biotechnol. Bioeng.* 25, 201–215.
- Tuovinen, H., Pohjolainen, E., Vesterbacka, D., Kaksonen, K., Virkanen, J., Solatie, D., Lehto, J., Read, D., 2016. Release of radionuclides from waste rock and tailings at a former pilot uranium mine in eastern Finland. *Boreal Environ. Res.* 21, 471–480.
- UNITE Community, 2017. UNITE Mothur Release. Version 01.12.2017. UNITE Community <https://doi.org/10.1515/BIO/587478>.
- Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., Kuske, C.R., et al., 2009. Three genomes from the phylum acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl. Environ. Microbiol.* 75, 2046–2056.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York, USA, pp. 315–322.
- Xie, X., Xiao, S., Liu, J., 2009. Microbial communities in acid mine drainage and their interaction with pyrite surface. *Curr. Microbiol.* 59 (2009), 71–77.